NATIONAL INSTITUTE FOR BIOLOGICAL STANDARDS AND CONTROL

Report
for 1978–80

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National Institute for Biological Standards and Control

(A World Health Organization International Laboratory for Biological Standards)

NATIONAL BIOLOGICAL STANDARDS BOARD

Director's Report to the Board on the work of the Institute from July 1977 to December 1980.

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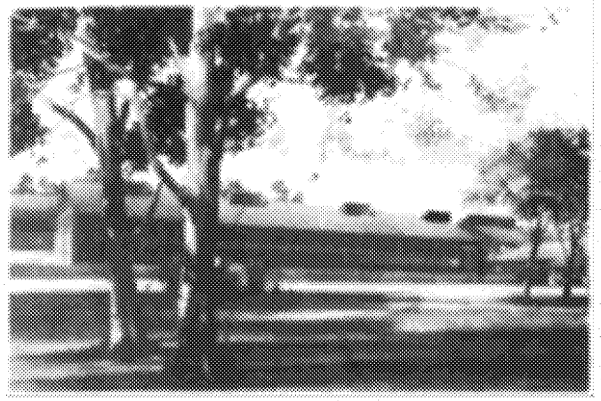
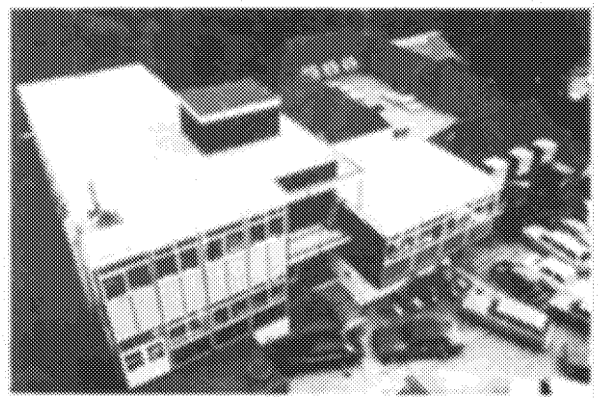
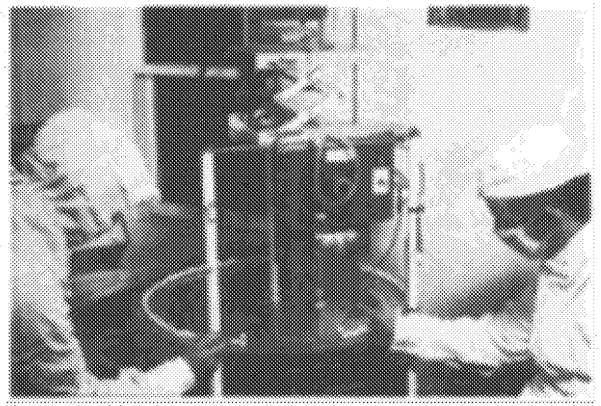


The new NIBSC Laboratories at Clare Hall, South Mimms. Artist's perspective of a laboratory wing, based upon architect's drawings.

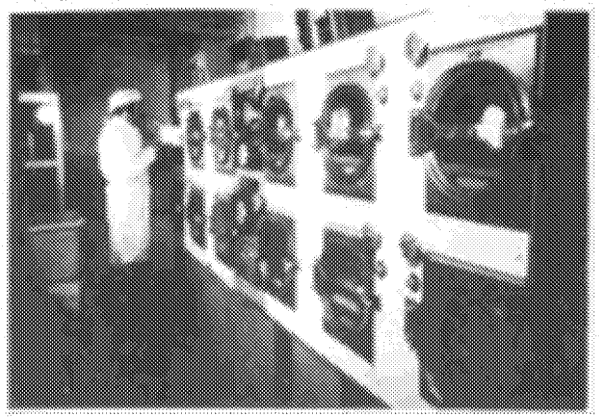


The Hormones Division Laboratories at Hampstead. The building behind it houses the canteen, workshops, electron microscopy and photography.

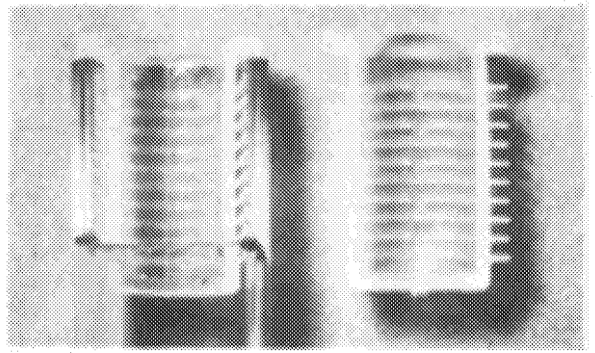
Mount Vernon House, on the Hampstead site of the Institute, houses the Administration Section.



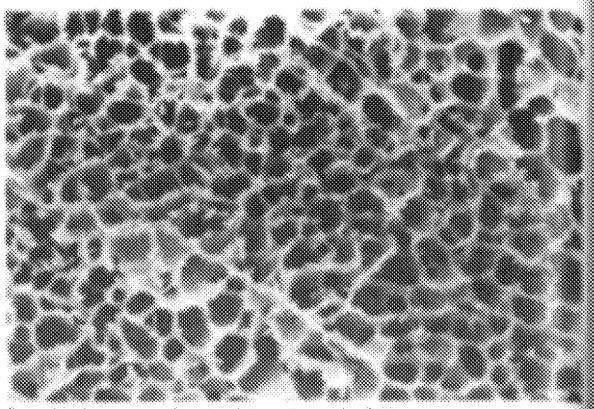
A prospective biological standard being distributed into ampoules in the Standard Processing Section.



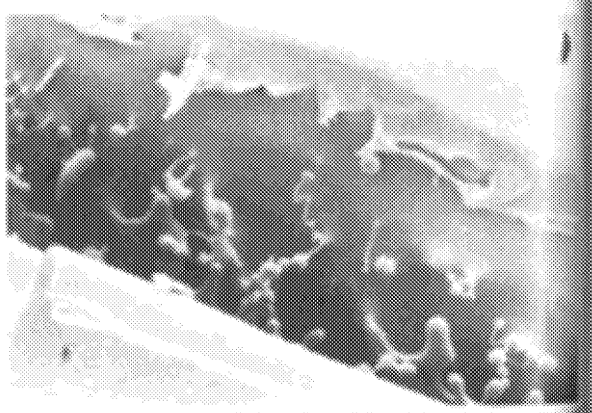
The chambers used for secondary desiccation of freeze-dried reference materials in the Standards Processing Section.



The polythene capillary-leak plug, used in the Standards Processing Section during preparation of reference materials. It permits secondary drying and the inlet of gaseous nitrogen after desiccation, but prevents significant loss of nitrogen during handling prior to permanent sealing of the ampoules by fusion of the glass.



Freeze-dried human serum. Scanning electron micrograph. × 2000.



Freeze-dried human serum immediately rehydrated before drying, showing precipitated salts and protein. Scanning electron micrograph. × 2000.

Contents

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G. Plimsaul

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Introduction

The National Biological Standards Board is responsible, on behalf of the Health Ministers of the United Kingdom, for the provision of biological standards, reference preparations and reagents, and for functions related to the control of biological substances used in human medicine. The Board came into being in July 1976 when it assumed its formal responsibilities under the Biological Standards Act of 1975. At the same time, the National Institute for Biological Standards and Control (NIBSC), the Institute through which the Board executes its functions, separated from the Medical Research Council to which it formerly belonged. An account of the antecedents and establishment of the Institute, and of the National Biological Standards Board and its responsibilities, was given in the Director's first report, together with relevant background information. For readers unfamiliar with this background, a summary is provided at Appendix 1 (page 121).

The scientific work of NIBSC is concerned with ensuring the purity and potency, and where appropriate safety, of biological products used in human medicine. Such products may be defined as those whose purity, potency and safety cannot adequately be evaluated by physical and chemical means alone. They include antibiotics, antisera, bacterial and viral vaccines, blood products, enzymes, hormones and other substances. Their potency can usually be assayed only by comparison with a biological standard preparation. Assessment of their purity and safety often requires examination not only of the final products, but also of source materials, which in many instances are living, as well as in-process samples.

The wide variety of different biological products necessitates a corresponding range of scientific disciplines at NIBSC. The Institute has five scientific Divisions: Antibiotics, Bacterial Products, Blood Products, Hormones and Viral Products; and five supporting Sections: Animal Services, Chemistry, Immunology, Standards Processing and Statistics. The work of these departments can broadly be divided into three: control, standardisation and research. The control activities involve advice on standardisation and control methods, the testing of manufacturers' products and the examination of production protocols. The work on standardisation requires the preparation of stable reference materials, the study of assay methods, and the collaborative study of prospective standards in order to determine their suitability and to agree an assigned unitage. Approved biological standards are then provided by NIBSC to manufacturers and other users in the United Kingdom and throughout the world. The Institute is a World Health Organization Laboratory for Biological Standards, and in this capacity its responsibilities relate particularly to International Standards for antibiotics and hormones, and also to certain blood products and vaccine preparations.

Research work provides the essential basis upon which these activities rest

The Director's first report concerned the work of the Institute and the Boar in its first year, from July 1976 to June 1977; this present report relates to th ensuing period to the end of December 1980. Despite the stringent economi restraints of these years, the period has seen the consolidation of NIBSC as a independent Institute and its growing national and international reputation a a source of scientific expertise and research in the standardisation and contr of biological medicines. The process of consolidation has been additionall interesting because it has taken place at a time when various fundament advances in molecular biology are being translated into practical bi technology. The pace of these developments has been rapid, and all bi medical organisations are needing to adapt in various ways to meet t challenges presented. Thus, recombinant-DNA technology, 'genetic engine ing', is already in use for the manufacture of insulin which is currently und going clinical trial in the United Kingdom. This technology is also bei explored for the preparation of other hormones and peptides, and of micr bial antigens that may yield new vaccines; many other applications are likely emerge. The technique of preparing hybridomas which produce monoclon antibodies provides another development of significance. These antibod have potential value for the purification of peptides, protective antigens a other substances, and they may also be exploited as therapeutic agents, f example, to control the immune response. When coupled with toxic molecul suitable monoclonal antibodies may prove of value in the treatment tumours, carrying the toxin specifically to tumour cell targets. They m further provide a supply of pure, specific reagents for use in standardisation of biological substances. In parallel with these biolog advances, methods of chemical analysis and synthesis are growing in th power to determine the structure of biological molecules and to manufact them *in vitro*, for use in medicine and elsewhere. The stimulating impact these and other developments has become clearly evident at NIBSC in period under review, and will be referred to in a number of places in t report, not least in relation to the control of the therapeutic products of b technology. There can be little doubt that these advances will have increasing effect on every aspect of the work of the Institute.

The work of NIBSC is presented here in the form of reports on each of departments, covering, where appropriate, work on control of biolog products, standards and standardisation, and research. The prelimi sections provide a more general review of the Institute's activities in the p three-and-a-half years, and draw attention to points that may be of gen interest in the departmental reports, where more detailed information ca found. A brief account is also given of the work of the National Biolog Standards Board and its committees; these include the project comm concerned with the design and planning of new laboratories for the Insti work on which had been in abeyance when the previous report was prepare

Control of Biological Products

The Board's Scientific Policy Advisory Committee completed in 1979 its first review of the control work of each NIBSC department. The conclusions affirmed that the control activities were generally satisfactory, but that expansion was required in certain areas, for example, in immunology and the blood products field. It has been possible to take on some of this added work, mainly by means of internal reorganisation. As an example, it has been possible to increase the amount of control work undertaken on blood clotting Factor VIII. The control tests on Factor VIII, used in the treatment of haemophilia, have been instrumental in securing significant savings to the nation's drug bill through correcting potency levels of imported batches which in many instances were found at NIBSC to be 20% lower than that claimed (page 29).

A new Immunology Section was established in 1979 to meet the need for greater attention to this subject at NIBSC. The Section incorporates the former Allergens Laboratory, and control of both provocation test and hypo-sensitising allergen preparations and associated research now forms part of the responsibilities of the new Section. The difficulties of controlling allergens are discussed on page 56, but it is worth referring here to the collaborative work programme on this subject in progress between NIBSC and the scientists of allergen manufacturers. The collaboration is seen by both the pharmaceutical industry and NIBSC as beneficial to their common aim of ensuring the quality of allergen preparations.

A third development has been the work on 'sweeps'—the testing of biological products taken from pharmacies and other points of use. Most control tests at NIBSC are done on manufacturers' products prior to release for sale, or on in-process samples. The Board was concerned, however, that deficiencies in biological products could remain undetected by such tests and be revealed only by tests on samples taken during the approved shelf life. For products not subject to tests prior to release, such checks might be the only means by which deficiencies could be exposed. With the help of DHSS staff, samples of all the licensed bacterial vaccines on the market in 1978 were collected and tested at NIBSC. Deficiences were found, and in consequence corrected, in the diphtheria component of diphtheria-tetanus vaccine. In the antibiotics field 'sweeps' have been completed on preparations of amphotericin, candicidin, erythromycin, neomycin, nystatin, and also on certain tetracyclines. Deficiencies were detected in a number of instances (page 19). Of special interest is the contamination of bulk nystatin with penicillin and erythromycin, which could be hazardous to patients sensitive to these antibiotics. These findings led, on advice from the Board, to the DHSS proposing an extension of the control over import of certain bulk biological

substances to include materials such as nystatin, gentamicin and also insuli

A major new field of control work has recently opened with the introducti of biological substances produced by 'genetic engineering'. A clinical tr certificate was recently granted by the United Kingdom Licensing Authority f the first 'genetic' product introduced for medical use—insulin. This material h the molecular structure of human insulin, and should therefore ha therapeutic advantages over the bovine and porcine insulins currently used f treatment of diabetes. The formulation of acceptable control requirements f the products of recombinant-DNA technology required careful considerati by the senior scientific staff of several of the disciplines represented at NIBS helped by outside experts. Such expertise was first secured by means of workshop on genetic manipulation held at the Institute in November 1979 which leading workers in the field from the United Kingdom and overs (page 91) contributed to an evaluation of the various factors involved producing a safe and effective drug by this new technology. Second, Dr P Greenaway, head of the Genetic Manipulation Laboratory at the Pub Health Laboratory Service's centre at Porton Down, has provided NIB with expert advice. In devising control requirements care is needed to see adequate assurance of purity and safety whilst avoiding the imposition over-cautious controls that could lead to unnecessary delays and depri patients of the potential benefits. The problems of dealing with 'gene products include the uncertain stability of the transforming plasmids. Chang in plasmid DNA could lead to the production by the transformed mic organisms of 'error' peptides, ie molecules differing only slightly in co position or structure from the intended product and which could pres unforeseen, potentially hazardous, biological properties.

The control procedures that were formulated, after much consideration, based upon those long applied to other products derived from living microb These depend upon the use of a seed-pool system, whereby a manufactu uses part of a frozen stock of approved, well-characterised micro-organis from which to culture each batch. For a transformed bacterium the seed wo normally comprise the host bacterium (*Escherichia coli* has been used so f and its transforming plasmids, the molecular structure of which has be established. At the end of the culture cycle evidence should be provided th the progeny remains unchanged in respect of both the host bacterium and transforming DNA. The purification process, by which the required produ separated from bacterial substances, including endotoxin and DNA, has t to be well controlled. Thus, bacterial endotoxin in the final product ca cause severe reactions in patients, and intact DNA sequences might hazardous. The specification of the product should demonstrate its molecu identity and integrity and, as far as possible, freedom from error peptides.

As experience of the processes and medicinal products of gen engineering is gained it may well prove possible, as with other biolog products, to relax the initial control testing requirements. In the case of hum insulin, first reports of the clinical trials indicate that a safe and effective d

drug has been introduced. Other medical drugs produced by recombinant-DNA technology will doubtless be introduced in the coming years. Human growth hormone produced in *E. coli* is already under study, interferons are being produced by the new technology, and manufacture of the protective antigens of a number of viruses and other micro-organisms is a field of active research with the goal of new vaccines against, for example, cytomegalovirus, hepatitis B and malaria.

Although, justifiably, recombinant-DNA technology has captured public attention, advances in chemical synthesis are also yielding biologically-active molecules, calcitonins, for example, that formerly had to be extracted from animal tissues. Interestingly, semi-synthetic human insulin has been introduced into clinical trial almost in parallel with the genetically-engineered product. The problems of control of such synthetic polypeptides are generally less complex than those of 'genetic' materials, although equal attention has to be given to the correct identity of the final product and its purity in respect of error peptides.

Among the scientific advances of recent years in the methods for chemical analysis, high-performance liquid chromatography (HPLC) has been of particular interest at NIBSC. This technique offers the possibility of rapid, discriminating analysis of mixtures of related substances, and sometimes of the identification of molecules, which in turn offers for some substances the possibility of cost savings from reducing and even eliminating the need for bioassay. HPLC has been extensively explored in the Chemistry Section and is finding increasing use in other departments. For example, the antifungal preparation nystatin is a complex mixture, the individual components of which vary in biological potency. The safe control of minimum quality therefore requires analysis of the composition of the mixture to supplement bioassay, and appropriate chromatographic test procedures have been developed to meet this need. For control tests on polypeptide hormones, work in the Chemistry Section has shown that HPLC can supplement (in the case of insulin, gonadorelin, calcitonins) or replace (oxytocin) bioassay. HPLC has also played a valuable role in examination of candidate materials for biological standards and of changes occurring in standards on storage. Its use is also being explored for characterisation of polysaccharide vaccines. The methodology is now being adopted in pharmacopoeial monographs and in WHO control requirements.

Licensing of pneumococcal vaccine in the United Kingdom in 1979 introduced a new field of work into NIBSC. The present vaccine contains the purified capsular polysaccharides of 14 pneumococcal serotypes. Although other polysaccharide vaccines, against *Haemophilus influenzae* type b and meningococci, have been licensed in other countries, the pneumococcal preparation is the first in the United Kingdom. Polysaccharide antigens behave differently from the familiar protein toxoids and whole-bacterial vaccines, and in the absence of suitable animal models, control has to depend very much on chemical analysis. The contributions of both the Division of Bacterial

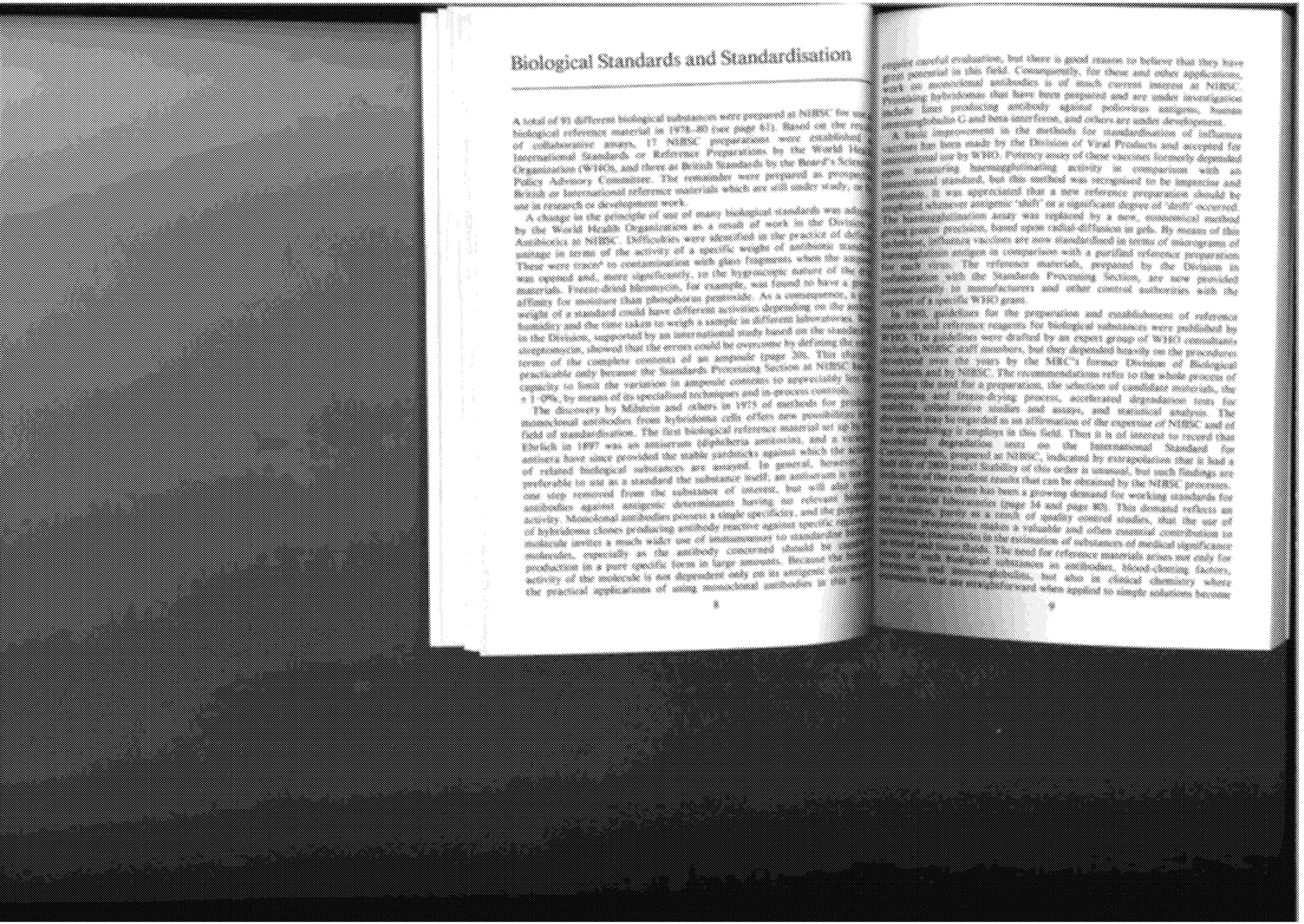
Products and the Chemistry Section were necessary in order to establish a
NIBSC the methods required for control testing. However, laboratory testing
cannot yet provide completely adequate evidence of the immunogenicity in
man of pneumococcal polysaccharides; a confirmatory test of the antibody
response in human volunteers is also required.

Three control problems for viral products have required special attention a
NIBSC in the past three years. The first concerns control of the safety of liv
poliovaccines. There had been an increasing failure rate of batches of type III
vaccine tested for neurovirulence at NIBSC, which caused difficulties over
vaccine supplies (page 40). Comparative testing showed that type III vaccin
virus has become increasingly neurovirulent with passage from Sabin's
original seed virus. Other work led to a basic improvement in the
neurovirulence test method (page 44) which is expected to secure inter-
national approval. Research work is continuing on the features that
distinguish vaccine virus from wild virus. At the present time it appears that
suitable seeds will be available to ensure adequate supply of live vaccine of
acceptable safety.

The threat posed to the United Kingdom by the spread of rabies acros
Europe has created pressure to ensure adequate quality control of rabie
vaccines. Fortunately, a much improved vaccine, manufactured in human
diploid cells, has recently become available which can safely be used for pre
exposure vaccination. However, control testing in respect of potency currentl
depends upon an *in vivo* test which is unsatisfactory owing to its imprecision
The test also poses difficulties for NIBSC, owing to the lack of laborator
facilities at Hampstead for handling the virus. Control testing and associate
research has therefore been done by staff travelling to use the containmen
laboratories of the PHLS Centre for Applied Microbiology and Researc
Porton Down, and related work has been carried out at PHLS, Colindal
with the help of Dr George Turner. The support of the PHLS in providin
NIBSC access to its laboratories is much appreciated. An in vitro test has no
been developed at NIBSC which should enable reliance on the in vivo test to b
reduced (page 47).

Interferon, a subject of current public interest in view of its anti-cance
potential, has presented a third control problem to the Division of Vira
Products. Control requirements had to be formulated for interferons derive
from transformed cells bearing some of the genome of a virus itself capable o
causing tumours, the Epstein-Barr virus. Moreover, the cells are stimulated t
produce interferon by infecting them with a second virus. It has been possibl
for the manufacturers to provide a high level of quality assurance as a result o
the purification that is possible by means of modern techniques—affinit
chromatography, for example. The manufacturing process can also be teste
for its power to exclude contaminating viruses or nucleic acids from th
product. Careful scientific evaluation of these questions, with the help of th
Viral Products Advisory Panel (page 90), has possibly established a basis fo
the control of products derived from biological systems, such as cell-lines, wil

neoplastic potential. The degree of quality assurance required of a biological product is a matter of risk: benefit judgement and, in the case of interferons, is inevitably influenced by the fact that they are first being studied in the treatment of human cancers. However, the experience gained with interferons will doubtless be of future value, since the products of cell-lines could well come into wider use. Thus, cell-lines are capable of giving a much greater yield of virus than primary tissue culture cells, and high yield in turn permits a high degree of purification and a less costly product. The control of these preparations is therefore of much international interest, in both the pharmaceutical industry and National control laboratories.



A total of 91 different biological substances were prepared at NIBSC for use
biological reference material in 1978–80 (see page 81). Based on the res
of collaborative assays, 17 NIBSC preparations were established
International Standards or Reference Preparations by the World He
Organization (WHO), and three as British Standards by the Board's Scien
Policy Advisory Committee. The remainder were prepared as prospe
British or International reference materials which are still under study, o
use in research or development work.

A change in the principle of use of many biological standards was ad
by the World Health Organization as a result of work in the Divisio
Antibiotics at NIBSC. Difficulties were identified in the practice of the
assays in terms of the activity of a specific weight of antibiotic stand
There were errors in communication with glass fragments where the
was opened and, more significantly, in the hygroscopic nature of the
materials. Freeze-dried laboratories, for example, were found to have a
affinity for moisture than phosphorus pentoxide. As a consequence, a
weight of a standard could have different activities depending on the
humidity and the time taken to weigh a sample in different laboratories.
in the Division, supported by an international study based on the streptom
streptomycin, showed that the errors could be overcome by defining the
terms of the complete contents of an ampoule (page 38). This change
practicable only because the Standards Processing Section at NIBSC h
capacity to limit the variation in ampoule contents to approximately
± 1·0%, by means of its specialised techniques and in-process control

The discovery by Milstein and others in 1975 of methods for pro
monoclonal antibodies from hybridoma cells offers new possibilities
field of standardisation. The first biological reference material set up by
Ehrlich in 1897 was an antiserum (diphtheria antitoxin), and a strong
antitoxin have since provided the stable products against which the
of related biological substances are assessed. In general, however,
preferable to use as a standard the substance itself, an antitoxin rather
one step removed from the substance of interest, but with also
antibodies against antigenic determinants having no relevant
activity. Monoclonal antibodies present a single specificity, and the
of hybridoma clones producing antibody reactive against specific
molecule invites a much wider use of immunoassay to standardise
molecules, especially as the antibody concerned should be
production to a pure specific form in large amounts. Because the
activity of the molecule is not dependent only on its antigenic
the practical applications of using monoclonal antibodies in this

require careful evaluation, but there is good reason to believe that they have great potential in this field. Consequently, for these and other applications, work on monoclonal antibodies is of much current interest at NIBSC. Hybridoma cell lines that have been prepared and are under investigation include lines producing antibody against poliovirus antigens, human immunoglobulin G and beta-interferon, and others are under development.

A major improvement in the methods for standardisation of influenza vaccines has been made by the Division of Virus Products and accepted for international use by WHO. Potency assays of these vaccines formerly depended upon measuring haemagglutinating activity in comparison with an international standard, but this method was recognised to be imprecise and inadequate. It was appreciated that a new reference preparation should be produced whenever antigenic 'shift' or a significant degree of 'drift' occurred. The haemagglutination assay was replaced by a new, commercial method using gamma precipitin, based upon radial diffusion in gels. By means of this technique, influenza vaccines are now standardised in terms of micrograms of haemagglutinin antigen in comparison with a purified reference preparation for each strain. The reference materials, prepared by the Division in collaboration with the Standards Processing Section, are now provided routinely to manufacturers and other control authorities with the support of a specific WHO grant.

No basic guidelines for the preparation and establishment of reference materials and reference reagents for biological substances were published by WHO. The guidelines were drafted by an expert group of WHO consultants including NIBSC staff members, but they depended heavily on the procedures developed over the years by the MRC's former Division of Biological Standards and by NIBSC. The recommendations refer to the whole process of preparing the bulk for a preparation, the selection of candidate materials, the processing and freeze-drying process, accelerated degradation tests for stability, collaborative studies and assays, and statistical analyses. The document may be regarded as an affirmation of the expertise of NIBSC and of the methodology it employs in this field. Thus it is of interest to record that the guidelines' degradation tests on the International Standard for Lymphokine, prepared at NIBSC, indicated by correspondence that it had a half-life of about 1 month. Stability of this order is unusual, but such findings are indicative of the excellent results that can be obtained by the NIBSC processes.

In recent years there has been a growing demand for working standards for use in clinical laboratories (pages 34 and page 80). This demand reflects an increasing awareness, partly as a result of quality control studies, that the use of a common preparation makes a valuable and often essential contribution to improved precision in the estimation of substances of medical significance even if such biological substances are antibodies, blood-clotting factors, hormones, viral immunoglobulins, but also in clinical chemistry where compounds that are neighbourhood when applied to simple solutions become

difficult when the substance is in plasma. Owing to limited resources, NIBS
has been able to give less support to this field than its importance justifies, bu
where possible help has been given, for example, in the form of scientif
advice, especially in the design and statistical analysis of collaborative assay
sometimes by making small trial batches of freeze-dried ampoules of
reference preparation for study, and in a few instances by providing a lar
batch of 3–4000 ampoules, for which a charge has recently had to be mad
Clinical laboratories are also increasingly using commercial kits as
alternative to bioassays which, in many cases, would be beyond the
resources. The manufacturer provides in his kit a set of carefully matche
reagents including a reference preparation. These diagnostic kits are not
subject to statutory control under the Medicines Act 1968, but the refere
materials provided in them should be calibrated in international units where
these exist. Requests for international standards by manufacturers
diagnostic kits have formed a recognisable part of the issues made by NIBS
and its predecessors MRC laboratories over the past decade.

Table 1 *Issues of Standards and Reference Preparations*

Year	No of WHO issues	Total No of issues
1977	7594	88 807
1978	7582	48 959
1979	9567	43 889
1980	8080	59 745

In 1980 (Table 1) just under 60 000 ampoules were issued from
Standard Processing Section (SPS), of which 8000 were samples of
national reference materials sent on behalf of WHO. The issues include
made to the various NIBSC departments for examination and study
process of characterising and establishing standards. The annual numbe
issues from SPS has fallen in recent years: in 1973–76 the number was
in excess of 100 000 each year and in 1975 it was 161 250. This fall refle
part a withdrawal from close involvement with certain clinical and diag
studies involving heavy demands upon issues of working standard
matched reagents, for example, for the assay of reproductive hormones
staff also exercise, as they have always done, control over the numb
ampoules issued in response to requests. Any limitations upon issue
however, to be imposed cautiously. On the one hand, stocks of
preparations need to be conserved to ensure the long-term availabi
identical standards, and to avoid the too-frequent effort of collabo
studies to establish replacements. On the other hand, standards are de
promote the adoption of agreed units of activity. The policy for

reference preparations is therefore a compromise between the aims of encouraging biological standardisation and of ensuring economy in the use of primary standards.

Research

The borderlines between research and the other activities of NIBSC are often difficult to define. In standardisation, for example, the preparatory work for an international collaborative assay may well involve research and development to establish reliable laboratory tests for the active substance, and this work merges into that of designing the collaborative assay. A large part of NIBSC control activities is inevitably taken up with ensuring the potency, purity and safety of new biological products, or of new processes for their manufacture, and the resolution of the associated scientific problems may again require research. The methods for control testing of established products are also studied. Such work often aims at developing simpler and more reliable tests, which may also reduce or eliminate animal tests—which are expensive and undesirable for humanitarian reasons. The separation between research and other work becomes clearer with longer-term studies, for example, to characterise chemically a biological substance or to establish the virulence markers of a virus from which an attenuated vaccine is being developed by the pharmaceutical industry. The separation is, however, most apparent in research unrelated to current and foreseen biological products. Thus a proportion of the research activities of the staff is independent of the former functions of the Institute, not least because the Board recognises that to maintain a staff of high calibre, able to deal with the difficulties of standardising and controlling new complex biological products, scientists must work in an environment in which the pursuit of research is fostered.

Many of the research activities of NIBSC are carried out in collaboration with scientists elsewhere. Links with the Medical Research Council, especially its National Institute for Medical Research, remain strong. The Hormone Division has worked closely in the parathyroid and growth hormone field with the Kennedy Institute (Dr J. Chayen and Dr Lucille Bitensky, WHO Laboratory for Cytochemical Assays). The Division of Antibiotics and Chemistry Section are frequently involved in joint studies with the laboratories of the British Pharmacopoeia Commission. The Division of Viral Products has an important grant-aided research project in progress on the genetics of polioviruses with the University of Leicester, and is also collaborating on the poliovaccine neurovirulence test with the control laboratories of Canada and the United States of America. In the present state of knowledge, it is probably true that no laboratory tests can entirely ensure the safety and effectiveness of any vaccine or, indeed, any biological medicine. The reasons are complex and include, in the case of vaccines, such problems as that of deciding which qualities of a vaccine account for immunogenic potency and safety, and assaying these qualities when they can, at least in part, be defined. Additionally, factors relating to usage influence the effectiveness of vaccines

such as the age of the recipients, the number of doses given, and in some instances the skill of the vaccinator. The immunological background of the population in which vaccines are used is also important. Consequently, the final test of a vaccine is its safety and effectiveness in the field. Part of the work of the NIBSC departments concerned with vaccines therefore involves collaboration in field studies, often in association with the Public Health Laboratory Service and, in particular, its Epidemiological Research Laboratory which has a strong interest in vaccine safety and effectiveness. There are numerous other research links between NIBSC and universities, hospitals, medical schools and other scientific laboratories, only a proportion of which can be referred to, at appropriate places, in the report.

Of the research activities in the *Antibiotics Division* the work on anti-tumour antibiotics, aimed at establishing means of assaying their anti-tumour effect, has been productive (page 22). Antibiotics such as bleomycin, for example, make an essential contribution to the excellent therapeutic results now being reported in leukaemia and Hodgkin's disease. Anti-tumour antibiotics which cannot be assayed by physico-chemical methods are currently ascribed a potency on the basis of their anti-bacterial activity, but this does not necessarily reflect their anti-cancer potential. Clinical dosage based on anti-bacterial activity is therefore liable to be unsatisfactory and possibly misleading. Evidence is accumulating, from work in many laboratories including those of the Division, that anti-tumour action is a consequence of DNA damage by the production of oxygen-free radicals. The ability of anti-tumour antibiotics to damage cell-free DNA may provide an assay of anti-tumour activity, and such a test has been developed in the Division, based upon release of malondialdehyde. At the present stage of development, the test is less precise than anti-bacterial assays and, as an interim measure, a search has been made for an indicator bacterial strain, the sensitivity of which to growth inhibition by anti-tumour antibiotics parallels their effect on cell-free DNA.

Chromatographic techniques, in particular the use of HPLC, have been developed for the analysis, characterisation and separation of anti-fungal antibiotics, neomycins and tetracyclines. The coil-planet centrifuge, developed in the Division with MRC staff, is being further exploited for the separation of peptide antibiotic complexes.

In the Division of *Bacterial Products*, studies on the toxicity of *Bordetella pertussis* vaccines affirmed that there is variation between different vaccines in the numerous toxic manifestations caused by this organism, but also that consistent differences of significance were not to be found (page 25). With the exception of the endotoxin, which is an accepted cause of pyrexia, the nature of any relationship between the various toxins of the organism and the rare neurological illnesses associated with the vaccine probably cannot be established in field studies. The best means of improving the safety of pertussis vaccine, whilst not reducing effectiveness, probably lies in basic work aimed at elucidating the nature of the protective antigens and at purifying these for use

as a vaccine. This has been the basis for much international research in the pa
20 years and, although steady progress has been made, the aim has yet to b
achieved. It has been decided to begin basic work on this organism in th
Division with the object of identifying protective antigens expressed on th
surface of the organism under *in vivo* conditions of growth.

Work referred to in the previous report on the potency assay of bacteri
vaccines used as immunostimulants and on the vibriocidal test for choler
vaccine potency was completed. Studies of BCG strains used in vaccines t
prevent tuberculosis have continued, although effort in this direction is to b
decreased; use of the vaccine has been stopped in some local authority areas i
the United Kingdom and general national use could come to an end.

Four areas of study are covered by the main research programme of th
Division of Blood Products (page 31). In the work on blood-clotting facto
and their inhibitors it has been shown that binding to phospholipids ca
protect clotting Factor VIII from inactivation, a finding which has possibl
clinical applications in the treatment of Factor VIII-resistant haemophili
Success in this work could lead to substantial cost savings for the Nation
Health Service.

In the fibrinolysis field the place of chromogenic peptide substrates a
indicators of fibrinolytic activity has been examined, and they have also be
found of use in the assay of antithrombin III in plasma. Assays are bei
sought for fibrin fragments which may indicate the severity of intravascul
coagulation.

The mechanism of platelet-vessel wall interaction is being studied in cl
collaboration with the electron microscopy unit—in this work the scanni
electron microscope has been especially valuable. A new finding is that t
normal endothelium is highly resistant to the effects of thrombin, wh
supports Dr D. P. Thomas's hypothesis that the main cause of veno
thrombosis in the presence of stasis is local generation of thrombin and n
endothelial damage.

Investigations of the mechanism by which heparin acts is the fourth ma
area of research in the Division. Heparin analogues and fragments which m
be of greater value clinically than heparin itself are being studied, in co
junction with the Chemistry Section. These substances are used in medicine
prevent thrombosis, but control of their potency is insecure because the ba
for their effects is not understood. The ideal preparation should prom
clotting but not cause haemorrhage, and low molecular weight preparations
heparin have been shown to possess such promising qualities in experimen
systems. There are important potential clinical applications of this work to
prevention and treatment of cardiovascular disease.

Part of the research work of the *Hormones Division* (page 36) is dire
to the detailed characterisation of peptide hormones, especially by analysi
their biological action. Preparations of certain pituitary hormones, f
example, consist of a complex mixture of chemically similar molecules, s
of which may be artefacts formed by degradation, but others represe

physiological and probably functional heterogeneity. The selection of material for an international standard to represent 'the hormone' therefore poses the fundamental question—of which molecule(s) should the standard be composed?

Another topic of research in the Division is the mechanism of action of peptide hormones on their target cells. Work with synthetic fragments of corticotrophin has shown that, uniquely, $ACTH_{4\text{-}24}$ induces steroidogenesis in adrenal cortex cells but without detectable cAMP accumulation; this could be evidence for two different types of hormone receptor and is the first observation of what may be a general phenomenon in the adrenal response to hormone. The desensitisation of target cells to their hormone, which is associated with certain physiological and disease states, appears to be due to the impaired function of a nucleotide binding protein (NBP) component in the hormone receptor system in cell membranes. Nucleated red cells desensitised to catecholamine had their responsiveness restored by their fusion with non-nucleated red cells containing fresh activitated NBP. This new model system helps to elucidate how a hormone regulates its own response.

A third topic of research in the Division includes the development of better assay methods. For example, preparations of human growth hormone extracted from human glands contain residual amounts of unwanted other pituitary hormones; simple methods are being developed to detect contaminant gonadotrophic hormones which must be controlled to ensure that dwarf children treated with growth hormone do not develop puberty precociously. Precise in vivo and in vitro assay techniques will also be required in the control of human growth hormone made by recombinant-DNA technology which will probably soon be on clinical trial. A new bioassay for calcitonins has been developed, based on the measurement of the cAMP response in renal cells. A comparison of results from this new bioassay and the current pharmacopocial method revealed the presence of an 'error' peptide.

An extensive international study of the methods of detecting pyrogens in pharmaceutical preparations is being co-ordinated. A promising new method, based on the effect of pyrogens on plasma zinc in mice, is under study as a possible economical replacement for the present rabbit test.

The *Division of Viral Products* maintains a strong research interest in the influenza virus (page 43). Reference was made in the discussion on standardisation to the improved methods for assay of the antigen content of influenza vaccines—an example of work which could equally well be included in this section under the heading of research. In 1980, and extending into 1981, Dr Frank Ennis, Head of the Virology Department of the Bureau of Biologics, USA, as a visiting worker initiated studies of the cellular response to influenza vaccines. Live vaccines stimulated natural killer lymphocyte activity, followed by release of interferon. Specific cytotoxic T-cell responses were also stimulated, and the significance of these effects in natural and vaccine-induced immunity is a field for further study.

Work on the poliovirus has also been given much attention in recent years.

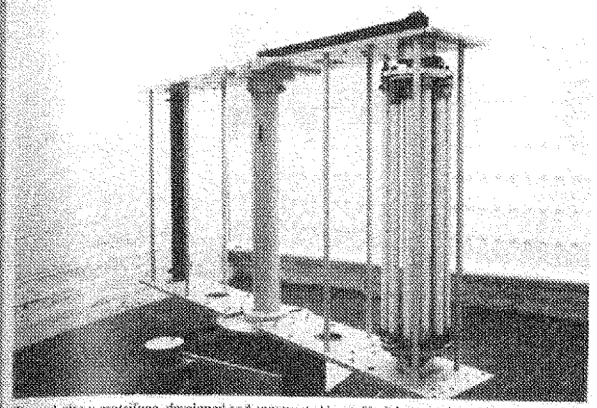
The supply of live vaccines has been uncertain owing to a variety of factor
including the limitations of the neurovirulence test in controlling vaccin
safety. Poliovaccine is, very rarely, associated with poliomyelitis in vaccinee
In consequence there has been renewed national and international interest
inactivated vaccines. Of much potential interest is the programme on th
genetic characterisation of polioviruses (page 45). With the use of
oligonucleotide maps it has been shown unequivocally that fatal cases o
paralytic poliomyelitis have been caused by viruses closely related to Sabi
vaccine strains. With support from an MRC grant, collaborative work with D
J. Almond (Leicester University) aims at cloning the genome of poliovirus typ
III strains. Of basic interest, this work could also lead to applications in th
construction of stable live vaccine strains, and in the production of purifie
antigen for use in vaccines. There is also the possible outcome of in vitr
techniques which could replace the costly in vivo neurovirulence test. A
autoradiographic zone-size enhancement technique has been developed fo
assaying the antigenic potency of inactivated poliovaccine. The method
considerably more sensitive and precise as well as being less costly than th
current *in vivo* methods, which it could well supersede, provided correlatio
with human immunogenicity can be demonstrated.

The Division has long had an interest in the standardisation of interfero
but the growing international studies of their clinical use, especially
treatment of cancers, has stimulated a research programme to characterise th
different interferons by use of monoclonal antibodies (page 47).

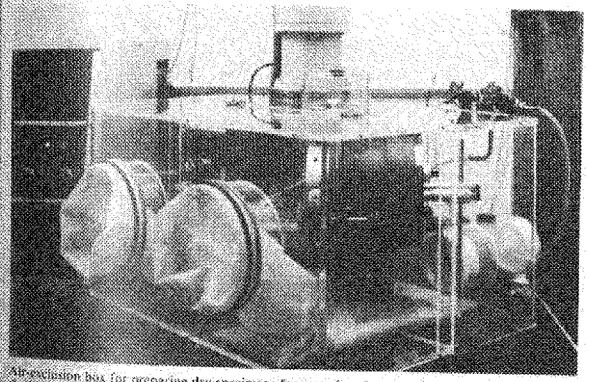
The study of polysaccharides and other carbohydrates continues to be
major interest of the *Chemistry Section* (page 53). Much evidence has be
obtained to show that heparins and other glycosaminoglycans prevent bloo
coagulation not only by action upon clotting Factor Xa.

HPLC techniques, and their applications to the analysis of biologic
substances, are a second long-standing interest of the Section. The method ha
successfully been applied to the separation of insulins and to analysis of
variety of peptides, including the assay of a combined preparation of oxytoc
and ergometrine. In carbohydrate analysis HPLC has been used for separatio
of glucose oligosaccharides. The analysis of larger molecules, such as certa
glycoprotein hormones, allergens and polysaccharides, is now being studied b
means of size-exclusion chromatography under HPLC conditions.

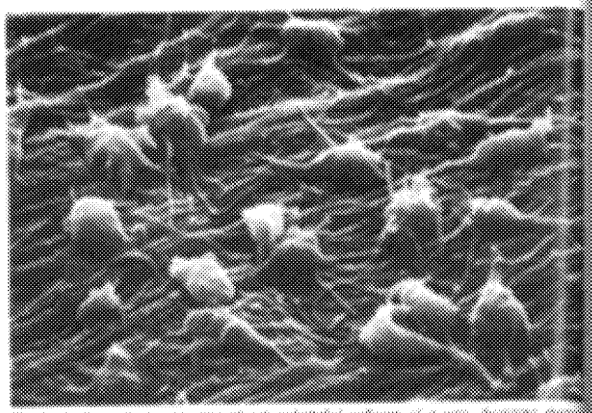
The *Immunology Section* has been much concerned with setting u
techniques for the production of monoclonal antibodies from hybridoma cel
lines (page 58). Monoclonal antibodies have been produced against c
membrane markers of human white blood cells and have potential applicatio
in the standardisation and control of these antibodies, which are now findi
clinical use, for example, in the treatment of leukaemia and graft rejectio
Promising results have also been obtained in the preparation of hybridom
synthesising antibodies against rat pituitary and hypothalamus cell membra
antigens. The application of hybridoma clones to the standardisation
allergen extracts is also being explored. In collaboration with the Division



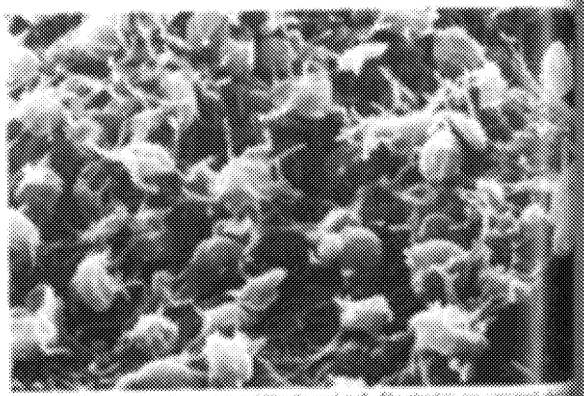
The cold-planet centrifuge, developed and constructed by staff of the Antibiotics Division, NIBSC, and the National Institute for Medical Research, Mill Hill. This apparatus is used for the separation of closely-related molecules.



Air-exclusion box for preparing dry specimens for scanning electron microscopy. Constructed by the Laboratory Engineering Workshop, NIBSC.



Blood platelets adhering to exposed sub-endothelial collagen on a vein. Scanning electron micrograph × 8250.



Blood platelets aggregated on damaged blood vessel wall. The platelets are activated and have long pseudopodia. Scanning electron micrograph × 6250.

viral products, monoclonal antibodies against poliovirus antigens have been prepared and characterized, and are proving of value in exploring the nature of the essential immunizing antigens of inactivated vaccines.

In the Standards Processing Section an examination is being made of the properties of bulking materials, which are often added to biological materials solely to protect them during the compacting and drying process (page 43). Lactose, which is commonly used for this purpose, has been shown to be capable of reacting with some biological substances and possible alternatives are being evaluated.

The research of the Statistical Services Section mostly derives from the problems encountered in its work on standardization and control (page 44). For example, in relation to current studies of the Listeria monocytogenes toxin and the endotoxin program, simulation studies have been carried out in the Section to help in relating the power of the test to that of the rabbit test for pyrogens. Again, work on human growth hormone has stimulated a reassessment of current methods of numerical analysis in the light of statistical theories of growth curves.

The theory underlying the accelerated degradation test, used to estimate the stability of reference materials, has been advanced, resulting in refinements of decisions on the number of ampoules to be studied, the temperatures at which they are incubated and the frequency with which they are assayed. A mathematical model has been developed for the clonal assay of human interferon cells; this work, done in collaboration with Dr R. Holliday (NIMR), has potential applications in the use of cell substrates but has also stimulated international interest owing to its relationship to the ageing processes of man and animals.

Antibiotics Division

The rate of innovation in chemotherapy shows no sign of slowing down. W
many new antibiotics represent fairly small alterations of existing molecules
major modifications or novel preparations appear on average about once
twelve each year. A proportion of these innovations concern antimicrob
substances which can be characterized by physical and chemical tests. M
many can easily be assayed biologically in units of activity agreed on
standard. Despite developments in physico-chemical analysis, few of the
'biological' antibiotics can yet be assayed by such methods alone—penicillins,
cephalosporins and chloramphenicol are the main examples. As
engineers' patents for established antibiotics run out, control of the produ
from new sources also demands attention, as has happened recently in the
of gentamicin and erythrin. The work required of the Division
consequently now diversified—and has indeed needed to respond
developments in two particular areas.

A principle often adopted in the control of biological products has
applied most clearly in the case of the antimicrobial drugs. A new antibiotic
first carefully evaluated and, upon licensing, the batch release procedure
instituted, whereby the manufacturer's protocols for each succes
production batch, together with samples of in-process materials and of
final product, are examined at NIBSC. The batch will be released by
Licensing Authority if NIBSC findings are satisfactory. This process
continues until it becomes evident that the manufacturer's laboratory
procedures are sound and that the material is consistently of adequate qua
Control by NIBSC will then be relaxed and, eventually, left to
manufacturer, with the safeguards that he is required to notify the Lic
Authority of any changes in source of materials or manufacturing process
is subject to inspection by the Medicines Inspectorate. The National Bio
Standards Board in 1977 decided, however, that there was a need period
to conduct tests on antibiotics (and other licensed materials) taken from
and other outlets—examples—to ensure that quality was being maintained
that it remained satisfactory during the products' shelf life (page 1). Th
led to added work for the Division, but the results, referred to in the sect
control work below, have clearly justified the exercise.

The second new area of work undertaken in the Division arose from
need to develop sound methods for potency assay and quality control
certain anti-tumour antibiotics, of which a small but growing number
growing of great value in therapeutic medicine. These studies are close
with the research work of the Division.

Control

During 1978–80, manufacturers' protocols and samples were received

361 batches of 14 different antibiotics. A number of irregularities were record
in laboratory testing procedures used by manufacturers, such as inadequate
control of assays, use of unsatisfactory reference standards and lack of
effective controls to test dry testing; all were corrected after discussion with
manufacturers.

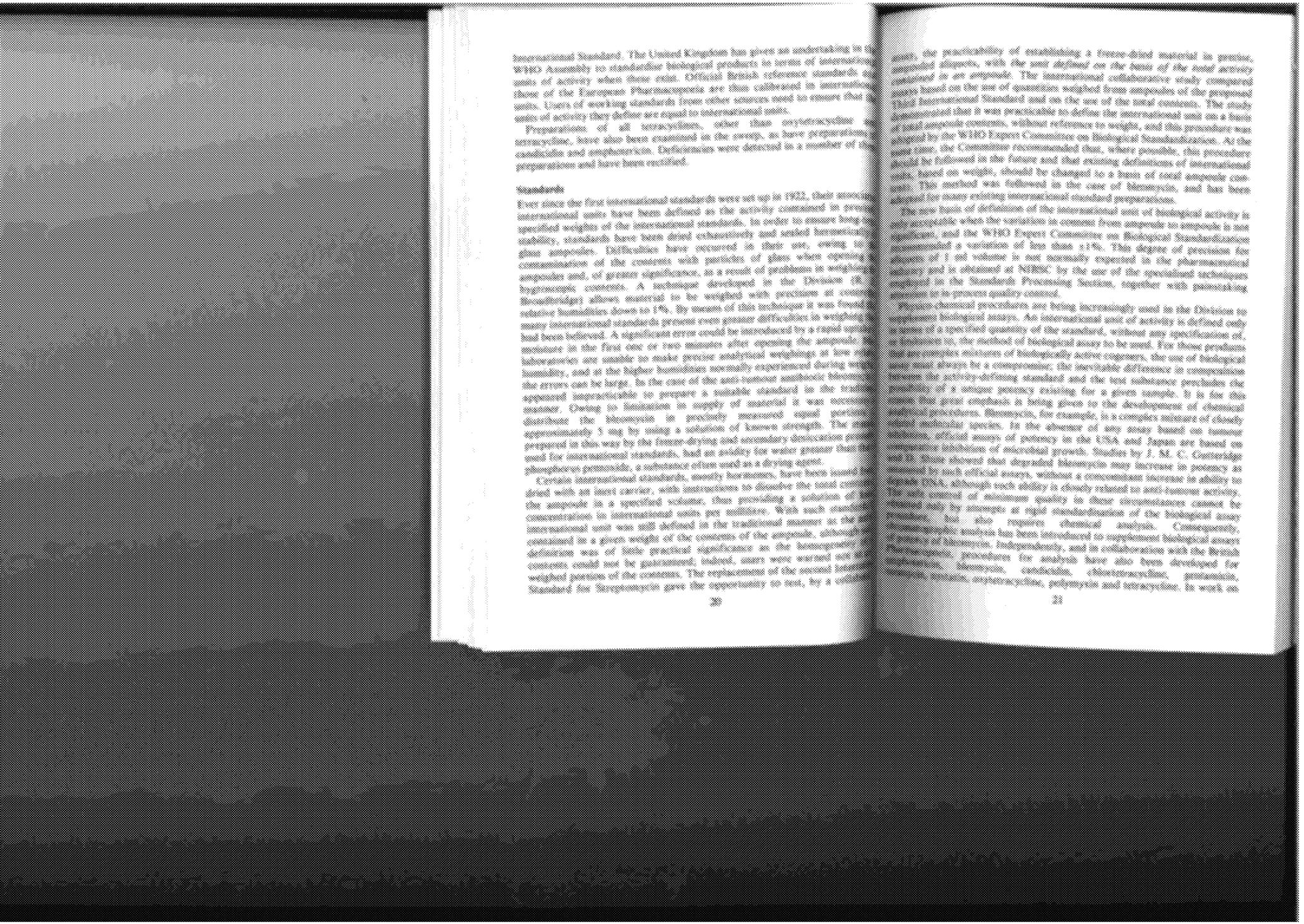
The expected introduction of newly developed antibiotics such as aztreonam
and netilmicin has required careful attention to proposals for specification of
minimum quality and safety in order to ensure that controls having consid
proved expeditiously once licences had been granted.

Control work on fluorocytosin involved studies to distinguish the inherent
impurity from any due to endotoxin contamination. Chromatographic
preparation of endotoxin from bituminite was achieved, allowing application of
the Limulus amoebocyte lysate test for endotoxin. At concentrations 10 times
higher than that required to pass the animal pyrogen test, bituminite batches in
which one were still free from detectable endotoxin.

The storage contamination of antibiotics from points of use, retail chemists and
hospital pharmacies, began in 1979 with preparations of streptomycin, obtained
by Mr. W. J. W. Price of Medicines Division, DHSS, with the collaboration of
regional hospital pharmacists and the Pharmaceutical Society's Inspectors of
Pharmacies. In many instances it was necessary to develop methodology to
extract the active ingredient from, for example, ointments and pessaries. A
number of manufacturers' preparations were found to be of inadequate
potency and steps were taken to rectify the deficiency. Five and one drops of
streptomycin presented special problems. Unusually low potency (eg 30% of that
stated on the label) was detected, and further investigations showed that
potency was inversely related to age of the product. Work in the Division, and
in manufacturers, traced the cause to a change in the British Pharmacopoeial
Ointment formulation which permitted a pH increase of approximately two units.
The lowered stability of neomycin at this more alkaline pH accounted for the
low potency. The pharmacopoeial specifications for these preparations are
now being revised.

Preparations of erythrin, one of the least stable antibiotics, were also
found in the first phase of the survey. An unusually high coverage was found
to be included in most formulations to ensure adequate potency at release and
until the expiry date; the acceptable coverage limit is still under consideration
jointly with BNFC and manufacturers. Bulk erythrin from one source was
found to be contaminated with penicillin and also what was probably
cephalosporin, and both materials from another source was contaminated with
tetracycline. Such contamination is potentially hazardous and has led to the
issue of advice from the Board, proposing the extension of control over the
purity of erythrin bulk biological materials to include penicillin and tetracycline.

Erythromycin products were also found to be deficient in potency. These
findings led to the fact that the United States Pharmacopoeia Reference
Standard, against which erythromycin entering the United Kingdom has
formerly been standardized, was approximately 8% more potent in terms of the



the peptide antibiotics, the coil-planet centrifuge, the development of which was referred to in the Director's first report, has proved of much value (by Newland). Although the first objective of this work is to control variation of proportional composition, ultimately it may allow a quantitative measurement of the different components which might replace the need for biological assay. In the case of oxytetracycline (A. H. Thomas) and gentamicin (P. de Rossi) HPLC procedures developed collaboratively in the Division may be accepted by the British Pharmacopoeia Commission as replacements for bioassay.

Physico-chemical analysis is also being applied to the choice of material for standards. When a first international standard is established for a new antibiotic, the choice of material is usually limited to one source by reason of patent rights. When a replacement standard is required, perhaps 15 or 20 years later, there may be many international sources of production; some may be licensed developments of the original manufacturing process and others may have been developed independently. The choice of a suitable replacement will be influenced by the behaviour of available materials under biological assay conditions, but a careful chemical examination may be more revealing. One sample of nystatin, of pharmacopoeial quality and high potency, was seriously considered as a replacement for the First International Standard for Nystatin. However, the material was shown by an HPLC procedure under development in the Division to have a composition which was quite atypical and would have been unsuitable as an international standard.

Based on work in the Division and collaborative assays the following reference preparations were established in the period under review: Bleomycin A2/B2 (IRP); Erythromycin (2nd IS); Candicidin (IRP); Streptomycin (1st IS); Tobramycin (IRP); and Nystatin (British S). Work is in progress on reference preparations for Amikacin, Bleomycin A5 and Sisomicin.

Staff in the Division played a major part in formulating the Guidelines for Quality Assessment of Anti-tumour Antibiotics which have been published by WHO.

Research

Interest has been renewed in the polyene antibiotics, which reportedly have immunological adjuvant and hypocholesterolaemic activities, and are synergistic with certain anti-tumour drugs. Work has therefore been undertaken attempting to correlate polyene structures with biological properties, including their effects on cell membranes. The results suggest that their action may be due to potassium ion release following membrane damage (A. H. Thomas). Chromatographic techniques, especially HPLC, have been developed to improve the quality control of these antibiotics.

Work has continued on the critical evaluation of the reactivity between general cell cytotoxicity, as demonstrated by the killing of bacteria, and the more specific anti-tumour property of ability to damage DNA (J. M. C. Gutteridge, D. Shute). The aim of this work is the development of laboratory methods for assaying anti-tumour activity of bleomycin and other anti-tumour

antibiotics to provide a secure basis for their therapeutic use in man. It was shown (1) that oxidative damage to the drug bleomycin leads to inactivation of its biological activities. This loss was closely paralleled in different strains of bacteria and in cell-free systems, and suggested a similarity of action for the drug *in vivo* and *in vitro*; (2) when one of the active components of the bleomycin complex was altered by a simple removal of one methyl group, significant changes in activity occurred, which highlighted problems of using bacteria as models for the cancer cell. These changes included a large increase in activity against *Mycobacterium smegmatis*, but loss of activity against *Bacillus subtilis*. Parallel studies of DNA damage monitored in a cell-free system by release of malondialdehyde confirmed that loss of activity as seen with *B. subtilis* was the more likely reflection of a change in anti-tumour activity of the drug complex. As the anti-bacterial assay in present use is unlikely to be matched in precision in the short-term future by assays of anti-tumour activity, selection of bacterial strains with a sensitivity to anti-tumour antibiotics which parallel *in vitro* damage to DNA appears to be a useful approach to a more satisfactory assay method. Evidence continues to grow, from work in the Division (J. M. C. Gutteridge with Dr B. Halliwell, King's College, London) and elsewhere, that the action of many anti-tumour antibiotics is to damage DNA by their ability to participate, in the presence of trace metal ions, in the formation of oxygen-free-radicals, the damaging effects of which cancer cells are less able to withstand than normal cells. J. M. C. Gutteridge is also collaborating with other workers (Dr D. Blake, Bath Hospital; Prof D. Armstrong, Oslo) on the role of free radicals in causing tissue damage in disease states of the retina and of joints.

A number of established procedures exist for the HPLC separation of both tetracyclines and neomycins, but these are all of a qualitative nature. The development and adaptation required to allow these procedures to be used quantitatively with a precision equal to that which can be obtained microbiologically is considerable, requiring very careful control of experimental detail, and is an important field of study in the Division (A. H. Thomas, P. de Rossi). It is possible that standard preparations of the individual components of heterogeneous, potentially toxic antibiotic complexes, such as candidicin, gentamicin, neomycin and polymyxin, will be required.

The use of the micro-measurements image analyser has been developed (R. A. Broadbridge) so that it can be employed for measuring zones of inhibition with very little contrast in density between areas of growth and inhibition, eg after short periods of incubation or when using *Pseudomonas aeruginosa* for the assay of carbenicillin. The precision of assays has been generally improved, allowing a saving in culture medium used.

Bacterial Products Division

The work of the Division has undergone a degree of re-orientation towards a biochemical approach to the problems of bacterial vaccines. Dr E. Griffith, an established worker in bacterial biochemistry, has joined the Division and with Dr K. Redhead is extending to *Bordetella pertussis* his previous studies of the effects of iron on bacterial pathogenicity. The physiological and morphological states in which bacteria act as pathogens *in vivo* often differ from those found in laboratory culture; an understanding of such changes in the whooping-cough bacillus is likely to improve knowledge of the antigens important in protection. Thus a successful microbial pathogen must possess the capacity to lodge and flourish in the human body, where the concentration of free iron, an essential element for bacterial growth, is extremely low. Certain pathogenic bacteria have been shown to possess the capability of adapting rapidly to a low iron environment, with the formation of surface molecules capable of chelating iron atoms and which may act as protective antigens. It is largely because these antigens remain unidentified that improved pertussis vaccines have not yet been developed.

A comparable biochemical re-orientation has occurred with the newly licensed bacterial polysaccharide vaccines that are necessarily controlled largely by analyses of the molecular characteristics of the polysaccharide antigens of which they are comprised. The need for physico-chemical methods has resulted in close collaboration with the Chemistry Section.

Control

In 1978–80, manufacturers' protocols and samples were examined from a total of 1364 batches of licensed bacterial products, including vaccines against anthrax, cholera, diphtheria, pertussis, pneumococcal infections, tetanus, tuberculosis and typhus, as well as tuberculin preparations and human and animal antisera.

The grant of a product licence in 1979 for the sale of a polyvalent vaccine for the immunoprophylaxis of pneumococcal infections necessitated the introduction of novel methods of quality control for a vaccine. Not only did the new vaccine contain more antigenic serotypes, 14 in all, than any vaccine previously marketed for human use, but all were polysaccharides of poor immunogenicity in laboratory animals and liable to spontaneous dissociation to small and inefficacious molecules. Further, the only certain method of potency assay known for the vaccine involved the immunisation and blood sampling of human subjects and the estimation of the vaccinees' antibody responses by a radioimmunoassay reputed to cost £15 000 for each vaccine tested.

The Division undertook the development of a double-diffusion technique

for the identification of each of the 14 polysaccharides in the final vaccine. This method was elaborated into a semi-quantitative procedure which provides a fair estimate of the quantity of each polysaccharide present. Simultaneously, an enzyme-linked immunosorbent assay (ELISA) was developed to replace the radioimmunoassay for the estimation of pneumococcal antibodies in human sera. The method was used for a vaccine potency assay conducted in a small group of volunteers from older NIBSC staff. Clear evidence of the practicality of an economic potency assay using ELISA was obtained, together with a demonstration of the efficacy of the tested batch of vaccine. The ELISA technique is now being used to investigate the antibody responses to pneumococcal polysaccharide vaccine of various groups of vaccinees who, because of disease or treatment of disease, may be considered at high risk from pneumococcal infection.

The Chemistry Section undertook a range of chemical and physical tests, repeating certain crucial tests such as gel filtration, and examining batches of the individual type-specific polysaccharides by other procedures such as optical rotation, chromatography and chemical analyses of specific monosaccharides. It is expected that a detailed profile can be assembled for each type of polysaccharide in each batch. The tests at present in use may require extension to ensure batch to batch reproducibility of the vaccine and, in due course, some of these tests may be replaced by others which are more specific and accurate. As part of the collaborative programme, longer-term studies are in progress to isolate sub-fractions of the polysaccharides, to determine their biological properties, and to try to correlate these with physico-chemical parameters.

An examination of bacterial vaccines collected from pharmacies and points of use was made in 1978–79. The vaccines included tetanus and diphtheria toxoids and pertussis vaccines and combined preparations of these antigens. Low potency was detected in diphtheria toxoid and subsequently other batches of the same vaccine were found to have an identical fault, which was remedied.

The Division now provides a sterility testing and microbial identification service for the Institute, mainly for the purpose of control testing (J. T. Watkins).

Standards

Studies in the Division led to the establishment during 1978–80 of the British Reference Preparation for Tetanus Antitoxin for the Flocculation Test, the 2nd International Standard for Pertussis Vaccine and the putative 1st British Standard for Pertussis Vaccine. A working reference preparation of human antisera was also set up for use in the titration of pneumococcal polysaccharide antibody.

Research

Investigations of the toxicity of pertussis vaccines (J. Hooker with P. Stevenson) involved tests of several different activities: adenylate cyclase

stimulation, inhibition of weight gain in mice, heat-labile toxicity, endotoxi
activity, histamine sensitisation and the induction of lymphocytos
hyperinsulinaemia, hypoglycaemia and pyrexia. These properties were foun
to be irregularly variable between different batches, and the range was simili
in British and North American vaccines. The seven-day weight gain test an
hyperinsulinaemia were found to be the most sensitive in detecting differenc
between vaccines and each production batch of pertussis-containing vaccin
for use in the United Kingdom is now tested for these properties, and for it
induction of hypoglycaemia and hyperinsulinaemia, as a check that significa
deviations do not occur. In the course of this work it was established that in
hyperinsulinaemia response caused by pertussis vaccine was not due
impaired insulin clearance, and was characterised by a subsequent refracto
period of one to two hours in which hyperinsulinaemia could not
reproduced.

Research on cholera vaccines explored the vibriocidal antibody response a
an alternative to the mouse protection test for the assay of vaccine poten
(A. W. Ford with L. Alterman). The vibriocidal response was found to
reproducible with a dose response to the vaccine which was correlated with it
protection response. The vaccines for use in field trials in Bangladesh we
examined in a variety of laboratory tests that might relate to potenc
including the vibriocidal response. The vaccines included toxoids of thr
different strengths, whole vibrio vaccine and various combinations of thes
The relative value of the different laboratory tests as indicators of potenc
should emerge when the field trial results become available. Work on choler
vaccines has been discontinued, both to make way for new work and in view
the low priority recently accorded to the use of vaccine in the internation
control of the disease.

Studies of BCG vaccine (T. W. Osborn with S. Clarke) have occupied ma
attention and have established the morphological variability of the differe
strains in current use. Whether this variability is phenotypic or reprodu
selection of genotypes from a mixed culture is under study. Although the us
of BCG in at-risk individuals may well continue, it is not unlikely that it coul
be removed from the national vaccination programme within a few yea
Work on BCG is therefore being wound down in order to give attention
more pressing questions.

Tuberculin tines have required examination (T. W. Osborn) in view of th
variability of the results that have been detected in their field use (Dr T. Lan
St Mary's Hospital, Paddington), traced in part to a variability of coating
the tines. It was shown that release of tuberculin in the skin required that t
tines be adequately coated and properly applied to the skin.

Collaborative field studies have been made on diphtheria and tetan
antitoxin responses (F. W. Sheffield and M. Melville-Smith, J. T. Watkin
These studies have, in general, provided valuable evidence that the vaccine a
vaccination schedules in use in the United Kingdom are continuing to prod
satisfactory immunity. It was shown that the booster dose of diphtheria-tox

vaccine advised at school entry was certainly needed if protective levels of diphtheria antitoxin were to be maintained in school children. The tetanus (and poliomyelitis) antibody levels were found generally to be less in need of reinforcement although the boosters did help to ensure continued immunity through schooling, and were of value in immunising children who had failed to be properly immunised in infancy. This work was facilitated by the introduction of the ELISA technique for the assay of tetanus antitoxin and the consequent elimination of much work with animals.

Previous studies, including those of E. Griffiths, have established that *Escherichia coli* is able to proliferate in the very low iron environment of the body by its phenotypic ability to form a high-affinity iron chelator, enterochelin, the t-RNA for which is switched on by low iron conditions. A research programme has now begun (with K. Redhead) to examine the effects of iron restriction on other pathogens which colonise surfaces, such as *Vibrio cholerae* and especially *B. pertussis*; at the same time the study of *E. coli*, which forms an ideal experimental model, is being continued. It is a reasonable hypothesis that *B. pertussis* growing in the respiratory tract differs from bacilli grown *in vitro* in the expression of secreted or surface molecules capable of chelating iron and acting as protective antigens.

Blood Products Division

Having been established as a new Division in 1976, the past three years have seen the consolidation and development of Blood Products as a separate department of the Institute, and also an element of expansion into new fields. A growing scientific reputation is reflected not only in its publications but also in the number of scientific visitors who have come for short periods of attachment for training or collaborative research. In 1980, the Division welcomed on long-term sabbatical leave Dr Anne-Marie Fischer, from Professor Josso's laboratory in Paris, Dr Klaus Hiller from Professor Gracff's laboratory in Munich, and Dr Neville Marsh from the Queen Elizabeth College, London.

In its review of the work of the Division in 1978, the Scientific Policy Advisory Committee (supported by Professor A. L. Bloom, Dr J. D. Cash, Sir William Maycock and Mr J. G. Watt) concluded that there was an urgent need to expand upon the excellent basis already established in order to undertake increased activities in several areas. These included further work on standardisation, control and research on blood-clotting factors, and control of the products of the National Blood Transfusion Service's plasma fractionation laboratories. Unfortunately the finance necessary to support such expansion has not become available, and only by means of an element of reorganisation of work within the Division has it been possible to accommodate some of these developments. An additional laboratory has been commissioned for the Division by means of reorganisation within the Institute, and is currently used particularly by visiting workers.

A Blood Products Advisory Panel (membership, page 89) was established to give advice in resolving scientific problems of standardisation and control. At its first meeting in December 1979, the Panel considered the standardisation of Factor VIII and advised that this required well-calibrated working standards, which are currently provided by NIBSC to manufacturers and also to the haemophilia clinical laboratories of the National Health Service. The clinical use was considered likely to grow, and this demand might possibly be met by the Blood Transfusion Service with help from NIBSC in the necessary collaborative studies for allocating potency. The Panel also considered the evaluation of prekallikrein activator in samples of plasma protein fraction and albumin. The standardisation of urokinase, which is complicated by the existence of two forms, high and low molecular weight, which behave differently in assays, was also discussed. A two-day scientific meeting was also held, in October 1980, to examine means of improving the standardisation of Factor VIII concentrate, and is further referred to in the section on control.

Control

The Division received protocols and samples of some 730 batches of blood products in the period under review. About half of these were of Factor VIII and 25% concerned albumin; the remainder included samples of heparin, Factor IX, streptokinase, urokinase and other products. A variety of defects were found including excessive amounts of prekallikrein activator in albumin samples. Control requirements for urokinase prepared from tissue cultures of renal cells were formulated with staff of the Viral Products Division.

An important recent development in control work has been the examination by the Division of protocols and samples of blood products manufactured by the plasma fractionation laboratories of the National Blood Transfusion Service. It is now possible to compare the material (albumin, plasma protein fraction, Factor VIII and Factor IX) produced by these National Health Service laboratories at Elstree, Oxford and Edinburgh with commercial blood products, and a fruitful dialogue with the Blood Transfusion Service manufacturers has been established. These products of the Blood Transfusion Service have been found to be generally of excellent quality.

The period under review has seen a continuing increase in the number of batches of Factor VIII controlled. Material is currently examined from five commercial firms and three Blood Transfusion Service laboratories. The amount of commercial albumin and plasma protein fraction received for testing has also shown a steady increase over the three years, and numerous batches of albumin from the Blood Transfusion Service are also now examined. There has been some increase in the number of batches of heparin that have been submitted for control purposes, while other drugs, such as streptokinase, urokinase and ancrod, have remained relatively stable in the number of batches received.

There has been a particular problem in the period under review in reaching agreement on potency estimates with the USA manufacturers of Factor VIII concentrate. This is of importance, not only to ensure that clinicians are provided with material of consistent potency, but also because of the cost consequences, as price is determined 'per unit'. NIBSC estimates of potency average between 20 and 30% less than those of the American companies. Approximately half of the total National Health Service usage of Factor VIII for the haemophiliac population is met by imported American products. It is hard to get precise figures, but a conservative estimate would be that somewhere in excess of 30 million units of Factor VIII are imported each year from the USA alone, representing a drug bill approaching £3 million. The problem has been that the American manufacturers have been using a Factor VIII standard prepared by the Bureau of Biologics, US Food and Drugs Administration, which has been a plasma standard inappropriate for assaying a concentrate material. The use of this standard also introduced an additional unnecessary step in the standardisation procedure for the American manufacturers. While reductions have been secured in claimed potency levels, in keeping with the results of NIBSC scientists, clearly this was inherently an

unsatisfactory situation. In October 1980 a meeting was held at NIBSC, attended by representatives of the US drug companies involved, together with representatives of the US Bureau of Biologics, other European and UK manufacturers, and clinicians who use Factor VIII. The problem was explored in some depth and it became clear that the main stumbling block was the interposition of an additional standard, namely an American national standard. It was generally agreed that if this intermediate stage of standardisation were omitted, and the US companies calibrated their house standard solely against the International Standard, a significant improvement would ensue. It was agreed at the end of the meeting that the Bureau of Biologics would no longer issue their own Factor VIII standard. It was also agreed that the WHO International Standard should be made available by NIBSC to US manufacturers for calibrating their house standards, and it is anticipated that there will be fewer problems in the future.

Standards

The main new International Standard established in the past three years was the first International Reference Preparation for Antithrombin III in 1979. Also established were the first British Standard for Blood Coagulation Factor VIII-Related Antigen, Human, for Immunoassay; the 7th, 8th and 9th British Standards for Blood Coagulation Factor VIII, Plasma, Human and a British Working Standard for Factor VIII Concentrate, Human. The WHO International Standards that are in most demand are those for Heparin and Factor VIII concentrate, and both of these will need to be replaced within the next two years. Discussions are already taking place with various interested scientists about how best to conduct the collaborative studies, and on the kind of standard that would be the most appropriate for these two important drugs.

There is a continuing and extensive need for the British Standard for Factor VIII, which is supplied as a working standard to the UK Blood Transfusion Service Plasma Fractionation Laboratories and also to those laboratories of the National Health Service involved with the treatment of haemophiliac patients. The Standard is replaced on average about once every 18 months. British Working Standards for Factor VIII and Factor IX concentrates have also been established and these are used by the production laboratories at Elstree, Oxford and Edinburgh and by NIBSC. By this means there is very little difficulty in agreeing with the potency estimates of the United Kingdom manufacturers of Factor VIII and Factor IX. On the international scene, the decision has been made in principle that it would be highly desirable to have a Factor VIII plasma standard as well as the existing Factors VIII concentrate standard, and a collaborative study is in progress.

There is a need for an improved plasmin standard, and the decision has been taken to replace the existing glycerol-based plasmin standard, which is inherently unstable, with a suitable freeze-dried plasmin. A collaborative study of a new plasmin standard is in progress.

There are ongoing problems in relation to agreement on the standardisation

of heparin and urokinase. It is of interest that both these drugs, which are of widely-differing character, share a common feature, that both high and low molecular weight forms are produced. The present standards are a mixture of the high and low molecular weight forms, and it is uncertain whether a mixed standard will prove satisfactory for calibrating material that is only of either high or low molecular weight. Discussion is taking place on this issue, and the Division is well represented on the various sub-committees of the International Society for Thrombosis and Haemostasis that considers these and related matters.

Research

The main areas of research in the Division fall into four broad categories: (1) the study of blood-clotting factors and their inhibitors, in particular Factors VIII and IX, and antithrombin III; (2) the biochemistry of the conversion of fibrinogen to fibrin and the removal of fibrin by fibrinolysis; (3) the platelet-vessel wall interaction, with particular reference to venous thrombogenesis; and (4) the mechanism of action of heparin and its analogues.

The work on blood-clotting Factor VIII has demonstrated that its binding to phospholipid can protect the factor from inactivation (T. W. Barrowcliffe, with G. Kemball-Cook, C. A. Eggleton and E. Gray). A proportion of haemophilia patients have Factor VIII inhibitors in their blood and their treatment is difficult, sometimes requiring very large doses of expensive Factor VIII. The possible applications of the protective effect of phospholipid are therefore explored. Successful work along these lines could lead to substantial cost savings to the National Health Service. The research into the relationship between phospholipids and blood coagulation is continuing with the aid of an MRC project grant. A number of collaborative workshops on the assay of clotting factors have been held which were helpful in identifying causes of major variations in results between different laboratories (T. W. Barrowcliffe and T. B. L. Kirkwood).

The binding of heparin to antithrombin III, the main physiological inhibitor of clotting, has been studied, and various commercially prepared antithrombins have been examined (T. W. Barrowcliffe, with C. A. Eggleton). It has become apparent, however, that heparin does not act solely on antithrombin III, and that other mechanisms also exist in the plasma for neutralising activated clotting factors. The ability of heparin and heparin analogues to release lipoprotein lipase has been a subject of particular study.

The development of new methodology for studying fibrinolysis has occupied much attention (P. J. Gaffney, with F. Joe and M. Mahmoud). The value of chromogenic substrates as indicators of fibrinolysis has been explored (with R. D. Philo) and it has been shown that they have certain advantages for the assay of antithrombin III in plasma and are of use in distinguishing high and low molecular weight urokinase. A new radioimmunometric assay was developed (with M. Spitz) which obviates the need to label either antigen or antibody. Improved assays are being sought for the measurement of the high-

molecular weight fibrin fragment (cross-linked X-oligomers) which may serve as a yardstick of the severity of intravascular coagulation and thrombosis.

A study is also being made (P. J. Gaffney, with F. Joe) of the functional defect and its location in variant fibrinogen molecules. Examination of polymerisation sites and thrombin binding sites of normal and variant molecules has been carried out using fibrin monomer immobilised on polyvinyl plates. It appears that the activities of fibrinogen associated with its polymerisation and thrombin-binding properties play an important part in the malfunction of variant molecules.

A detailed study is in progress (D. P. Thomas, with R. E. Merton) of the mechanisms of experimental platelet-vessel wall interactions. In close collaboration with the electron microscopy unit (D. Hockley, with C. Baigent) an ultra-structural study has been carried out of the response of the venous endothelium to various forms of stasis, mechanical injury and high local concentrations of the enzyme thrombin. In contradiction to published results using tissue culture, it has been shown that the normal endothelium is highly resistant to the effects of thrombin, either in the presence or absence of stasis. This is an important finding in relation to the pathogenesis of venous thrombosis. D. P. Thomas had suggested previously that the prime cause of venous thrombi developing in the presence of stasis is the local generation of thrombin and not endothelial cell damage. The fact that thrombin is able to produce stasis thrombi without damaging the vessel wall is entirely consistent with the well-recognised finding at autopsy that the veins are usually normal in patients with deep vein thrombosis.

The mechanism by which heparin and heparin analogues prevent experimental venous thrombi has also been studied (D. P. Thomas, T. W. Barrowcliffe, with R. E. Merton). An ideal antithrombotic agent should prevent clotting without promoting haemorrhage, and significant progress to this end has been made in the past three years. Using specially prepared low molecular weight heparin, it has been found possible to prevent venous thrombi with levels that have essentially no effect on overall clotting; this observation may have important clinical implications.

Hormones Division

Scientific developments are now taking place which are liable to affect the work of the Division profoundly. These include, for example, synthesis of hormonally-active polypeptides of small to moderate chain length for clinical use; exploitation of genetic engineering in micro-organisms to synthesise larger polypeptides; the discovery of new growth factors and the endorphins; the realisation that several peptide hormones act within the nervous system as well as on the gastro-intestinal tract; and advances in the methods by which the concentration of these and other hormone analytes can be assayed in clinical specimens. The Division's work programme, discussed below, already reflects a number of these developments, but resources will have to be carefully managed to ensure that the important therapeutic products of these developments can adequately be standardised and controlled.

Control

Human insulin prepared from amino-acid chains produced in *Escherichia coli* by genetic manipulation has presented the most important new problem. This product is the forerunner of other peptides and proteins made by recombinant-DNA technology, and the principles of their control had to be formulated. This was an endocrinological problem only in so far as this first medical product of genetic engineering happened to be a hormone. It also has to be borne in mind that insulin is often injected two or three times daily for years on end, in some 3–5% of the population. The approach to its control was considered in detail by the scientific staff of NIBSC, helped by advice from a microbial geneticist, Dr P. J. Greenaway (CAMR, Porton Down), and by a workshop held at NIBSC in 1979 (page 91). It was concluded that the new control measures should be based firmly upon the established principles of control of medicinal products from live organisms (page 4). The reports of the first clinical trials of the new human insulin appear highly promising.

In addition to human insulin made by genetic engineering, semi-synthetic human insulin manufactured by chemical modification of pure porcine insulin has also required control. Recently introduced analytical methods, such as HPLC, have been of great utility in evaluating the purity of these and other products.

Apart from the introduction of insulin identical with the human hormone, there has been an almost complete changeover to manufacture of high purity insulins of porcine and bovine origins. Prior to this changeover, an analytical survey of conventional insulin preparations had revealed a high proportion with other protein components and with marked deamidation.

Work in the Division, with the Chemistry Section, using techniques such as electrophoresis, electrofocusing and HPLC, has exposed the relative impurity

of certain synthetic peptide products, such as tetracosactide, human and salmon calcitonins and desmopressin; in a number of instances work in collaboration with manufacturers' scientists has effected improvements in quality of these products.

During 1978–80, some 850 batches of manufacturers' hormone products intended for medical use were examined in the Division, including 582 batches of insulin. Findings in the Division led to withdrawal from the market or non-release in the case of batches of insulin, corticotrophin and human growth hormone. Control of human growth hormone has required much attention, and a research programme aimed at improving and simplifying its control testing is in progress (page 38).

Standards

Many of the procedures for the preservation of biological reference materials and their characterisation by international, or national, collaborative study were first developed in the former MRC Division of Biological Standards, and improved over some 20 years, more recently by the work of various NIBSC departments including the Statistics Section and the Standards Processing Section. The principles based on this long experience formed a major part of guidelines for the preparation and establishment of reference materials published by WHO in 1978 for international use.

The need for reference standards in endocrinology is evident in three main areas; for control of potency of therapeutic products; to facilitate research work on endogenous hormones and in clinical pathology, enabling laboratories to relate their results in equivalent units; and for standardisation in the assay of hormones in clinical laboratories. The demand in the last area is growing rapidly, as the importance of standardisation is becoming more appreciated, often as a result of quality control schemes among pathology laboratories. Moreover, many laboratories require working standards for those hormones that are heterogeneous, scarce, unstable or difficult or costly to standardise. Although NIBSC resources are, in the main, appropriate to the provision not of working standards but of primary standards intended for use mainly in the control of therapeutic products and in research, it has been possible to give some help in this growing field. Thus, working standards have been prepared for the assay of human thyrotropin and prolactin, but collaborative work with the Supra-Regional Assay Service has supported the development of specialist regional assay laboratories which, as the system becomes established, can adequately be served by NIBSC providing primary standards to one or more central laboratories which can prepare their own working standards.

Based to a considerable extent upon work in the Division, the following WHO reference materials were established during the period July 1977 to December 1980: 1st IRP of Placental Lactogen, for Immunoassay; 4th IS for Oxytocin, for Bioassay; 1st IS for Arginine Vasopressin, for Bioassay; 1st IS for Lysine Vasopressin, for Bioassay; 1st IRP of Calcitonin, human, for

Bioassay; 1st IRP of Chorionic Gonadotrophin, for Immunoassay; 1st IRP of Prolactin, Human, for Immunoassay; 1st IRP of LH, Human Pituitary, for Immunoassay; 1st IS for Desmopressins; 2nd IRP of FSH/LH, Human Pituitary, for Bioassay; 1st IRP of Gonadorelin, for Bioassay.

Other work on hormone standards in the period under review includes the following examples:

Insulins Samples of highly-purified bovine and porcine insulins are under study to replace the less pure materials that have hitherto served as national and international standards and which are not suitable for standardising the new purified insulins. A standard of human insulin is also now required and work has begun to set up appropriate British and international preparations (D. R. Bangham and A. F. Bristow).

Tetracosactide A five-year programme of work on a prospective standard for tetracosactide (1–24 corticotrophin) for bioassay, prepared by Ciba-Geigy, has now been completed in collaboration between that firm and NIBSC (P. L. Storring).

Corticotrophin Since there is no readily available source of highly purified human ACTH for the preparation of an IRP, the possibility has been examined of isolating ACTH from a side-fraction of the procedure used to extract growth hormone from human pituitary glands (P. L. Storring and Prof W. Butt).

The International Standard for Corticotrophin, which was prepared by the Division in 1959 and is of porcine origin, was shown, by extrapolation from accelerated degradation tests, to have a half-life of 2800 years—a striking example of the high stability which is sometimes possible when peptides are carefully freeze-dried in appropriate conditions.

Human parathyroid hormone Assays of parathyroid hormone are vauable in the differential diagnosis of clinical hypoparathyroidism, for which a number of commercial assay kits are marketed. The provision of a standard has been particularly difficult because of the extreme scarcity of supply of the human hormone, which is obtainable only from rare adenomas removed at operation. A research standard was set up three years ago, with international collaboration, and this has been widely used. In 1980 0·2 mg of purified extract was donated (Prof C. Arnaud) and has been distributed into 4000 ampoules and freeze-dried. An extensive collaborative study, in which this material was compared by bioassays and radioimmunoassay with the research standard, local standards and with sera from selected patients, showed that it was suitable to serve as an international reference preparation (J. M. Zanelli and R. Gaines-Das).

Human chorionic gonadotrophin International Reference Preparations of Human Chorionic Gonadotrophin (hCG), and of the α and β subunits, were respectively assigned units by WHO following an extensive international collaborative study (P. L. Storring, D. R. Bangham and R. Gaines-Das). In this study the new IRP of hCG was compared, by a wide range of in vivo and in vitro bioassays, receptor assays and various immunoassays, with coded

samples of serum and urine of early and late pregnancy, and with the International Standard for hCG for Bioassay. This latter standard is used for bioassay of preparations of hCG for clinical administration; the new IRP is used to calibrate kits to detect pregnancy and for monitoring patients for choriocarcinoma.

The International Standard (established in 1964) is an impure material which also contains α and β subunits of hCG, whereas the new IRP consists of much more highly purified material. The unit assigned to the latter was based on results of *in vivo* bioassays, since the hormone 'hCG' consists of those glycoproteins which have high biological potency in classical *in vivo* bioassays. Hitherto, the sensitivity of pregnancy test kits has been calibrated by comparison with the International Standard; the replacement of the latter with the new IRP has led to the need to recalibrate certain assay kits with the IRP of hCG and/or the IRP of hCG β subunit.

Work is also in progress on reference preparations for: arginine vasopressin, corticotrophin, follicle stimulating hormone, human growth hormone, human urinary kininogenase, insulin C-peptide, luteinising hormone, porcine pancreatic kininogenase, prolactin and secretin.

Research

Research in the Division has three main themes:

(1) *The identification and characterisation of peptide hormones*

It is now recognised that several of the pituitary hormone extracts contain a number of molecules which are chemically closely similar to each other. Some of these forms are known to be artefacts produced by the extraction or storage of the material, but several hormones in simple extracts from fresh tissue appear to be naturally heterogeneous. In the case of peptide hormones such as hGH, prolactin, parathyroid hormone and gastrins, the forms may be different pieces of the same original molecule. Insulin, for example, is a portion of the precursor molecule proinsulin. But the glycoprotein hormones TSH, FSH, LH and chorionic gonadotrophin show intrinsic heterogeneity, and the chemical nature and the purpose of the heterogeneity is not yet fully understood. This poses the fundamental question: what molecules, in what relative proportions, comprise 'the hormone'? This question must be considered each time a hormone preparation is selected to serve as a reference material. In the case of the gonadotrophins FSH and LH, several highly purified preparations made by leading laboratories have been assayed by a variety of *in vivo* and *in vitro* bioassays, receptor binding assays and immunoassay. The potencies of these extracts have been shown to differ by as much as five-fold in different assay procedures. Following their separation by isoelectric focusing, individual components of 'FSH' and of 'LH' have been further assayed using very sensitive *in vitro* cell bioassays and radioimmunoassays (P. L. Storring, Y. Mistry, with Dr E. Diczfalusy and Dr A. Zaidi, Stockholm). Results show that 'FSH' and 'LH' in all preparations, and in the extracts of fresh pituitaries, are heterogeneous. It has yet to be studied whether each

individual form of such hormones has differing biological actions and whether their secretion as mixtures provides a means of subtle modulation of hormone action.

Attempts are being made to isolate a factor causing the release of corticotrophin (ACTH) from cells in the pituitary, analogous to the releasing factors for TSH or the gonadotrophins. Although prompt release of corticotrophin has been observed, it has been difficult to isolate any one active peptide. By using very gentle, rapid methods, an extract of frozen porcine hypothalamus has been obtained which shows very marked activity in the detection system used (assay of corticosterone released from adrenal cells treated with medium from pituitary cells exposed to the extract). Claims have been made that vasopressin is a prime releaser of corticotrophin, but the vasopressin in the extract studied accounted for less than 1% of the releasing activity of the whole extract. Work is in progress, using HPLC, to separate the active components in the extract and in blood (D. Montague, D. Schulster and A. F. Bristow).

(2) *Mechanisms of action of peptide hormones on target cells*

Corticotrophin (adrenal corticotrophic hormone, ACTH), a linear peptide of 39 amino acids, causes accumulation of cAMP in adrenal cortical cells when it induces them to produce corticosterone. Using synthetic fragments of ACTH (from Dr Schwyzer, Zurich) and dissociated adrenal cells, it was shown that $ACTH_{5\text{-}24}$ induces steroidogenesis with no observable cAMP accumulation. Moreover, $ACTH_{6\text{-}24}$ more effectively inhibited cAMP accumulation elicited by both $ACTH_{1\text{-}39}$ and $ACTH_{5\text{-}24}$ than steroidogenesis. This suggests two new concepts of hormone target-cell mechanisms: (1) that cAMP is not necessarily an essential component of this target cell response to its agonist hormone; and (2) that on adrenal cortex cells there are two types of hormone receptor for ACTH, one cAMP-dependent and the other acting by another unknown mechanism (D. Schulster, M. Salmon and S. Fatih).

Recent studies suggest that rapid phosphorylation of target cell membranes may be a component of the response to peptide hormones acting via cAMP stimulation. Using bovine adrenal cortical cell membranes it was shown that within 10 seconds ACTH induced the phosphorylation of several membrane proteins, and only a small amount of cAMP was produced when extensive phosphorylation occurred. Thus either cAMP is highly compartmentalised within the region of the membrane where adenylate is contiguous with protein kinases, or ACTH-mediated membrane protein phosphorylation can occur by mechanisms independent of cAMP (A. Bristow and M. Salmon).

Previous work has shown that hormone-dependent cAMP accumulation is mediated by receptors which are mobile within the membrane lipid matrix. During cell fusion such receptors can migrate and become coupled with the adenylate cyclase of a foreign cell type. This work has been solely concerned with the coupling of receptors to adenylate cyclase. A peptide hormone binding to its target cell receptor activates the N coupling protein (or nucleotide regulatory protein) and cAMP. Human mature red cells have N protein but have lost their cAMP system and have no β-adrenergic receptors.

Turkey red cells have adrenergic receptors, N protein and cAMP. If they are exposed to too much isoproterenol they become desensitised and fail to respond. Experiments have shown that if such cells are fused with human red cells (using Sendai virus) their β-adrenergic responsiveness is promptly restored. This suggests that the deficiency of N protein has been remedied by the supply from the red cell, and that N protein is an important regulator of the response of a target cell to its peptide hormone (M. Salmon, C. Wilson, D. Schulster).

(3) *Assay methodology*

Human growth hormone 'Growth hormone' is probably a mixture of similar peptides which stimulate increase in growth of long bones and muscle, although the particular biological effects of each peptide are not known. The potency of clinical preparations is assayed in a test which depends on increase in body weight. This assay is imprecise and costly and an in vitro alternative is being sought. First a study was made using cytochemical procedures *in vitro*, responses of which showed a quantitative relationship to a previous injection *in vivo* of hGH (G. Limbrey and D. R. Bangham, with Dr J. Chayen and Dr L. Bitensky, Kennedy Institute). The increase in activity of the enzyme 6-glucose phosphate dehydrogenase (6 GPD) in epiphyseal cartilages, liver and skin were selected for study. The effects of hGH on the skin and liver were so rapid as to suggest that they were direct effects of the hormone and not mediated by somatomedin peptides produced in response to the hGH.

Another control problem with preparations of hGH is the need for a suitable test to show that they contain an acceptably low level of contamination with other pituitary hormones, such as gonadotrophins, which accelerate sexual maturity, thyrotrophin, which could induce hyperthroidism, and vasopressin, which could cause water retention. Bioassays for these hormones are laborious, costly and insensitive, and would not detect denatured forms which could theoretically lead to auto-immune disease when they were injected repeatedly for months on end. Although radioimmunoassay procedures are sensitive enough for this purpose, they are so difficult to standardise as to make them unsuitable for formal control tests. With the aid of a research grant from DHSS, simple immunoprecipitation procedures have been developed for use as control tests (G. Limbrey and D. R. Bangham).

Calcitonin and parathyroid hormone A new *in vivo* bioassay for calcitonins has been developed depending on the increase in cAMP as the response signal in the kidney. This assay is much more rapid and less costly than the conventional bioassay of calcitonin, based on calcium levels in rats (J. M. Zanelli, B. Rafferty and P. M. Jones). By comparing results of bioassays involving these two different responses, an error peptide which was active in one system but not the other was detected in a preparation of synthetic salmon calcitonin.

Various other metabolic events in cells can be measured by cytochemical procedures. Using fastidiously controlled conditions, identified biochemical changes can be stained quantitatively and the intensity of the stain in individual cells measured with a microscope densitometer. With such

procedures it is possible to identify and analyse the nature and timing of response of cells and tissues exposed to hormones or other substances. It has been shown that parathyroid hormone (PTH) activates different enzymes in three distinct parts of the kidney convoluted tubules. Using the G6PD response, intact (1–84) PTH causes two sequential phases of enzyme activity. The fragment 1–34 PTH, which is active *in vivo*, elicits only the early phase, and the oxidised hormone, which is inactive *in vivo*, elicits only the later stage. Results of such studies with various portions of the hormone suggest that cells possess a second site in one or more receptors for the carboxy-terminal end of the hormone, hitherto regarded as biologically inert—perhaps a feedback mechanism concerned with control of hormone effect and certainly independent of the cAMP-mediated biological response.

The 6GPD response has been used to develop an extremely sensitive bioassay for intact PTH in plasma. With this method it has been shown that in patients with pseudohyperparathyroidism with high levels of immuno-reactive peptide, the level of biologically active PTH is in fact normal (C. Arber, J. M. Zanelli, with Dr J. Chayen and Dr L. Bitensky).

Detection of pyrogens Contamination of biological products with bacterial endotoxins (pyrogens) gives rise to practical problems, of protecting the safety of the patient, of the cost of the steps necessary to minimise the risk of contamination, of testing for pyrogens, and of the loss of expensive or scarce products if they fail the statutory pyrogen test.

Endotoxins elicit a general body systemic response, of which fever is one component. The present official test for pyrogens involves measurement of the body temperature in groups of rabbits after the test specimen has been injected intravenously. Rabbits have to be housed in special conditions, they are costly, and the test is difficult to standardise. Preliminary experiments suggested that the depression of plasma zinc concentration was another characteristic of this systemic reaction capable of development into a reliable test in mice (R. E. Hartley, S. Boobis and D. R. Bangham). It is almost as sensitive as a commercial Limulus amoebocyte lysate (LAL) test for bacterial endotoxins and, unlike the latter, it has the great advantage of being sensitive also to pyrogens from Gram-positive bacteria. Evaluation on this promising test (S. Boobis and B. E. Stenning) is being made with the various coded endotoxins included in a large collaborative study comparing the LAL test and the statutory test in rabbits, organised in association with WHO (R. E. Hartley, D. R. Bangham and M. V. Mussett).

Viral Products Division

This Division, which forms the largest of the NIBSC departments, secured a modest expansion in 1978, with the recruitment of an additional scientist and technician to undertake work on rabies and hepatitis. Rabies has spread steadily westwards across Europe since World War II, and in time will probably reach the Channel coast of France. Because of the threat to the United Kingdom posed by this advance, high priority was given to research on rabies vaccines. Although this work has been hampered by the absence of a Category A laboratory in which to handle the virus, collaboration with the Lister Institute, until it closed, and with the PHLS laboratories at Colindale and Porton Down, has enabled progress to be made. In the case of hepatitis, the introduction of vaccines against hepatitis B virus was foreseen, and the acquisition of the necessary skills and development of techniques for their control and standardisation were recognised as tasks of high priority.

With the approval of the Board, the Division was accorded the title in 1980 of World Health Organization Collaborating Laboratory for Viral Reagents. The title can be regarded as formal recognition of the influence of the Division's scientific staff on international standardisation and control.

Among the numerous scientific problems with which the Division has been involved in the period under review, the control of live poliovaccine has required great attention. A number of manufacturers' batches of poliovaccine, mainly of type III, were found at NIBSC to be excessively neurovirulent and were in consequence not released. This safeguarded the public against the increased risk of vaccine-associated poliomyelitis, but also led to difficulties in maintaining a supply of vaccine of acceptable neurovirulence. A research programme was undertaken which demonstrated that type III vaccine virus had undergone an increase in neurovirulence during passage in tissue culture. This and other findings stimulated a search for a safer seed virus from which to prepare batches of vaccine, and also to a detailed study of the nuerovirulence test. This work, further described on page 44, has led to a fundamental improvement in this essential test, with the added advantage that the new method requires the use of fewer animals.

Control of the immunogenicity of influenza vaccines is difficult owing to the antigenic variability of the causative viruses—sometimes a number of new epidemic variants are observed in a single winter. A major improvement has been developed in the Division, whereby the protective antigen can be quantified in a gel diffusion test by comparison with a characterised reference preparation.

Control

The Institute is greatly helped in formulating control tests and requirements in the complex field of viral products by its Viral Products Advisory Panel

(page 90), which was joined in 1979 by Professor N. Grist (University of Glasgow), Professor H. Stern (St George's Hospital) and Professor N. P. L. Wildy (University of Cambridge). Regrettably, the death of Dr R. J. C. Harris, a valued panel member, occurred in 1980. Dr Harris was formerly director of the Medical Research Establishment, Porton Down. The Panel met four times in the period under review and considered the poliomyelitis neurovirulence test, control requirements for interferons and the preparation of a hepatitis B surface antigen standard.

Owing to the potential hazards of virus vaccines all batches manufactured in the United Kingdom, and those imported, are examined in the Division, both by checking the manufacturers' protocols and testing final products, bulks and in-process samples, as well as the cell substrates in which the vaccine viruses are cultured. For certain vaccines an added means of ensuring safety derives from use of the MRC-5 and MRC-9 strains of human diploid cells for culturing the vaccine virus. These cells were developed and characterised in the division (and its MRC predecessor); the work on the MRC-cells was completed in 1979. By all available test methods these cells are free from extraneous viruses and other abnormalities and can be provided to manufacturers for use as acceptable substrates for culture of vaccine virus. In the period under review, a total of 189 samples of MRC-5 cells and 11 of MRC-9 cells have been issued, in many cases for the purposes of vaccine production. At the same time, 234 cultures of human diploid cells were received from manufacturers for control tests. MRC-5 cells are now in use for the routine manufacture of poliomyelitis, rabies and rubella vaccines, and for the preparation of experimental herpes virus vaccine.

The standardisation of killed influenza vaccines has been put on a sounder footing by the introduction of reference preparations of influenza virus haemagglutinins specific for individual serotypes (page 42). Control of these vaccines now includes the assay of antigen content in microgrammes of haemagglutinin antigen. During 1978–79 a total of 63 lots of inactivated influenza vaccine antigens were examined at NIBSC, the high number reflecting antigenic variations of the virus in these years. Of these vaccine batches five were found to be deficient in one respect or another.

During 1978–79, several preparations of poliovirus vaccine were found to be unacceptably neurovirulent and were not approved for release.

Interferons manufactured in the United Kingdom were released for clinical trial in 1979. Although the balance between the potential benefits and risks of interferon products is influenced by the fact that the treatment of cancers is the main subject of these trials, their standardisation and control has required great care, not least because they are produced from sources such as lymphoblastoid cells, which are unusual even in the biological field. Products tested and released for clinical trial in human cancers, derived from lymphoblastoid cells, have been of uniformly high purity and potency. The definition of interferon dosage depends upon the use of a suitable standard preparation; in this respect, standards established by earlier collaborative work at NIBSC have proved invaluable.

The routine testing of inactivated cell culture rabies vaccines has continued. By the mouse protection test several batches of vaccine have been found low in potency. This test has been found to give variable results with wide confidence limits, and progress has been made in establishing a more secure test for controlling the effectiveness of this important vaccine (page 47).

Monitoring of measles, rubella and mumps vaccines, especially for potency and stability, has continued. A manufacturer's batch of live vaccine represents the progeny, in very great numbers, of an approved seed virus known to be capable of yielding a safe and effective vaccine. Some, or all, of this progeny could possibly have reverted to virulence. Safety testing of live vaccines depends in large measure on demonstrating that the vaccine virus has not reverted and is identical in its properties with the seed virus. By this criterion, and other tests, these vaccines have generally been of acceptable quality, but loss of potency was detected in two batches of rubella vaccine, which were consequently withdrawn from use by the manufacturer. The potency loss was attributed to contamination of the ampoule stoppers by butylated hydroxy-toluene; routine testing of the stoppers for this substance has now been adopted by the manufacturer.

Standards

A major improvement in standardisation of influenza vaccines has been developed in the Division. Formerly, the main protective haemagglutinin antigen of these vaccines was assayed in haemagglutination units by comparison with the international standard. This method is imprecise and rests on a biological activity, agglutination of red blood cells, which does not closely relate to immunogenic activity. The new method, based on immunoassay of haemagglutinin antigen by single radial diffusion, gives improved precision and is applicable to inactivated vaccines of all types; it has been adopted by WHO for international use. The technique requires the preparation of a purified reference antigen whenever a significant change occurs in a prevalent virus strain; with the aid of a specific WHO grant suitable reference antigens are provided by NIBSC for the international control of inactivated influenza vaccines.

The Division has collaborated with the PHLS in several studies on the immunogenicity and acceptability of new influenza vaccines and the information obtained has been of value in helping formulate the DHSS annual recommendations for immunisation against influenza. Influenza vaccines of greatly improved purity have been manufactured in recent years. These 'surface antigen' vaccines contain only the haemagglutinin and neuraminidase antigens of the virus, and have been found to have good immunological properties and generally to be of lower reactivity than purified whole-virus vaccines.

New reference vaccines for poliomyelitis virus types II and III have been identified, and work is continuing to establish a suitable type I reference. These reference viruses provide the yardsticks of safety against which the

neurovirulence of batches of poliovaccine is tested. It is advantageous if these reference preparations are agreed internationally, and to this end the Division has been involved in collaborative studies with the control laboratories of other countries and with WHO.

A reference preparation for hepatitis B surface antigen has been prepared and studied in an international collaborative study organised from NIBSC with the support of an advisory group (page 91). The material used, heated plasma from a human carrier, was provided by Dr D. S. Dane. The results of the study are at present being analysed, but the preparation should be of value in the control of the new hepatitis B vaccines and in the the standardisation of diagnostic tests for hepatitis B surface antigen.

Research

INFLUENZA

Antigenic analysis (G. C. Schild, J. Oxford, J. M. Wood, with R. W. Newman and T. Corcoran.) The protective effect of influenza vaccines cannot reliably be forecast and may well be influenced by the specificity of the haemagglutinin antigens present. Work on the antigenic characterisation of influenza viruses has therefore continued, using conventional serology and, more recently, monoclonal antibodies (with M. Spitz). The information obtained on antigenic relationships between viruses has also contributed to the development of a simplified and scientifically relevant nomenclature system adopted by WHO for international use.

Improvements have been sought in methods for measuring antibody responses to influenza infection, and the relationship of these responses to protection has continued under study. A method based on radial-haemolysis in gels has been found generally more sensitive and reliable than conventional haemagglutination-inhibition tests, and has been used to analyse the specificity of the antibodies elicited in volunteers by vaccines. Attempts are in progress to define more clearly the parameters of the antibody response to vaccination which best correlate with immune status. It was found that vaccines evoked predominantly anti-haemagglutinin antibodies which were reactive within a subtype, together with strain-specific antibodies directed against previously encountered influenza viruses of the same subtype. These findings provide a clue to the limited protective efficacy sometimes associated with influenza vaccines during periods of antigenic drift.

Cellular immunity In 1980 scientists in the Division, led by Dr F. A. Ennis, a visitor from the US Bureau of Biologics, in collaboration with the MRC Common Cold Research Unit, Salisbury, and the University of Sheffield, investigated in volunteers the lymphocyte responses to live and inactivated vaccines (F. A. Ennis, G. C. Schild, with Yi-Hua Qi, D. Riley). Natural killer lymphocyte activity was significantly increased shortly after infection, and was associated with the appearance of interferon, which may have induced the killer cell activity. Vaccines also evoked a virus specific, HLA-restricted,

cytotoxic T-cell response; these lymphocytes appear to be important in animals for recovery from influenza infection. The stimulation of cellular immunity may be a desirable quality in vaccines and it will be interesting to determine the persistence of this lymphocytic response following vaccination and whether it correlates with protection against challenge.

Genetic studies (J. Oxford, G. C. Schild, with T. Corcoran, R. N. Newman and D. Major.) New influenza A viruses with completely changed haemagglutinin and neuraminidase surface antigens (termed antigenic shift) arise possibly by genetic recombination between the influenza A viruses which exist in nature in human or animal hosts. In addition, the genes coding for the haemagglutinin and neuraminidase antigens are unusually labile, and these antigens demonstrate partial changes (antigenic drift) with high frequency. Considerably less is known about the effects of genetic variation in the six remaining viral genes which code for internal components and non-structural proteins. Determination of the gene sets responsible for virulence is directly relevant to the development and control of live attenuated vaccines. By genetic characterisation of a series of recombinant viruses of known virulence for man it was established that virulence may involve several of the viral genes, including that coding for haemagglutinin. This work has relevance to control of live influenza vaccines which require to be immunogenic and non-virulent, and also incapable of reverting to virulence.

Extensive genetic, biological and biochemical differences have been detected among influenza A and B viruses prevalent within a single epidemic period. Even in outbreaks in a single town or school both influenza A and B viruses have been detected with marked structural differences in their proteins and RNAs. A proportion of H1N1 and H3N2 viruses examined had a temperature sensitive (*ts*) phenotype. Since *ts* properties have been associated with attenuation and have been used to select strains for use as live vaccines, it will be of interest to determine which genes are responsible for the *ts* phenotype, and to investigate the comparative virulence of these viruses. The studies have been extended to include analysis of individual genes by T_1-oligonucleotide mapping and RNA hybridisation techniques which would be expected to detect single mutations. A number of viruses isolated over the previous 40 years have been included in the study, which may enable an evolutionary pathway of individual genes to be established.

POLIOVIRUSES

Neurovirulence tests (P. Reeve, L. F. Taffs, V. Seagroatt, with S. Marsden, J. Ridley.) In part as a result of collaborative studies over the past three years between NIBSC, the US Bureau of Biologics and the Bureau of Biologics, Canada, an improved test has been established. Previous methods were imprecise and could not readily distinguish vaccines near the borderline for neurovirulence acceptability. In the revised method, the spread of virus specific lesions in the central nervous system from vaccine virus is compared

with that of a reference virus in the same test; previous methods did not routinely include a reference virus in each test. The studies showed that the lesion scores were relatively independent of the dose of virus given, and estimates of neurovirulence could be obtained using only one or two input doses of virus, enabling a reduction in the numbers of monkeys used compared to previous procedures. There was good agreement between the relative activities of reference and test vaccines in tests performed in the three collaborating laboratories. Recommendations for the use of the technique, the statistical analysis of data and suitable reference viruses have been made to WHO, and it is anticipated that the revised test system will be adopted for international use.

Genetic characterisation (P. D. Minor with S. F. Jeffries.) The use of T_1-oligonucleotide mapping has made it possible to identify poliovirus strains unambiguously. Unequivocal evidence was provided for the first time that viruses isolated from the central nervous system of fatal cases of poliomyelitis associated with recent vaccination were closely related to Sabin vaccine strains.

A collaborative study (supported by an MRC research grant) has started with Dr J. Almond, Leicester University, with a view to cloning the genome of selected type III poliovirus strains. This work should lead to an understanding of the nature of the attenuation of the vaccine virus and, in consequence, the replacement of the costly in vivo neurovirulence test by in vitro methods. It could also progress to the production of poliovirus proteins as a new form of inactivated vaccine, or to the construction of stable, live vaccine strains by recombinant-DNA technology.

Antigenic analysis (G. C. Schild, M. Spitz, J. M. Wood, D. I. Magrath, with A. John.) Renewed interest in inactivated poliovaccine has arisen because there have been difficulties in the supply of live vaccines and because advances in biotechnology now enable the large-scale commercial production of vaccines of improved potency and purity. Such vaccines have been subject to extensive field trials in developed and developing countries and have been found to be satisfactorily immunogenic in children following two doses. Laboratory tests for controlling the potency of these vaccines are at present unsatisfactory, standardisation being hampered by incomplete knowledge of the antigenic structure of polioviruses. The Division has therefore embarked on studies to improve understanding of poliovirus antigenic determinants and their relationships to virus structural proteins. Classical studies suggested that the essential immunising antigens are associated with the intact virus particle (D antigen) rather than empty capsids (C antigen). Investigations in the Division suggest a more complex situation. Animals immunised with purified C antigen particles were found to develop neutralising antibody almost as frequently as those immunised with D antigen. With the Immunology Section several hybridoma cell lines have been developed with specific reactivity for antigens of poliovirus type III strains. Analysis of virus harvests using these reagents has revealed four populations of particles. These include two distinct

populations of rapidly sedimenting (RNA containing) particles with sedimentation values of 55S and 130S with D and C antigenic activity respectively, and two populations of slowly sedimenting empty particles (80S and 70S), also with D and C antigenic activity respectively. Although D and C particles differ in their major antigenic determinants, they have been found to possess some determinants in common. Moreover, among a panel of 12 monoclonal antibodies to poliovirus type III, those with the highest virus neutralising activities reacted equally well with D and C particles, whilst three monoclones with specific D antigen reactivity were inefficient in virus neutralisation. These findings suggest that antigenic determinants relevant to neutralisation are present on D and C particles and that both may contribute to the immunising potential of vaccines.

Monoclonal antibodies have proved to be of great value in the precise characterisation of poliovirus strains. A potentially important finding is that a collection of eight type III viruses, all designated 'Saukett' and used as seed for the manufacture of inactivated vaccines, were antigenically heterogeneous. Such differences may help to explain discrepancies which have been reported between antigenic content and immunogenicity in man for different vaccines. Other potential uses of monoclonal antibodies are as reagents in immunoabsorbent column chromatography methods for the commercial preparation of vaccines, and for immunoassay of antigenic content of vaccines.

Assay (J. M. Wood, G. C. Schild, with U. Dunleavy.) Inactivated poliovirus vaccines are conventionally assayed in tests of their antigenicity in small laboratory animals. These tests are imprecise and variable from laboratory to laboratory. Autoradiographic zone size enhancement (ZE) techniques, based upon single-radial-diffusion, have been developed to provide an alternative *in vitro* assay system. The poliovirus ZE test depends upon a competitive affinity for antibody of vaccine antigens and radioactively-labelled D antigen preparations. Studies to establish standard antigens for use with this method are in progress, employing vaccines which have been extensively studied in human volunteers, in order to make correlations between *in vitro* antigen content and antigenicity in man.

Field studies (D. I. Magrath, with A. John.) In surveys carried out with the Avon Health Authority and the PHLS, serum samples from children aged 7–16 years have been studied. Over 80% of the children had antibody to all three virus types. Five per cent of children lacked antibody to type III virus. Some 200 children were given a booster dose of either inactivated or live, attenuated vaccine. Antibody responses to a single dose of live vaccine were good in children with little or no antibody, but less good when the pre-boost antibody titre was between 1/10 and 1/32. Following inactivated vaccine high levels of antibody were induced in more than 95% of children irrespective of the pre-boost titres. In the United Kingdom primary vaccination against polio achieves persisting adequate levels of antibody in a high proportion of children, but live vaccine administered at school leaving is less effective than inactivated vaccine in boosting antibody levels.

INTERFERONS

Interferons are currently being studied in clinical trials against various human cancers and virus-mediated diseases. At present, preparations used clinically are mixtures of different interferon molecules, but single molecular species may prove more effective in certain situations. Therefore, it is necessary to provide effective purification procedures for each interferon species and there may be as many as 20 of these. Monoclonal antibodies have proved valuable for purification purposes, and a programme of research at NIBSC aims to develop and characterise such antibodies to alpha-interferon (lymphoblast origin), beta-interferon (fibroblast origin) and gamma-interferon (T lymphocyte origin) (A. Meager, G. C. Schild, M. Spitz). The monoclonals will be of value in the characterisation of interferon mixtures, and for controlling the potency of preparations in quantitative radio-immunoassays as alternatives to the conventional antiviral assays.

RABIES

Studies to develop a more reliable protection test are in progress. An in vivo test for the antigen content of rabies vaccines is under study, and an assay for rabies glycoprotein antigen based on single-radial-immunodiffusion (SRD) has been devised (M. Ferguson, G. C. Schild, with P. J. Yates). Plots of zone area against relative antigen concentration were linear, and the method was found to be of suitable sensitivity for in vitro potency assays of inactivated, cell culture rabies vaccine. Studies to determine the relationship between the antigenic content of vaccines in SRD tests and their immunogenicity in man and animals are in progress.

HERPES VIRUSES

There is growing interest in the development of vaccines for a number of herpes viruses, including Epstein-Barr virus, cytomegalovirus (CMV), varicella zoster (VZV) and herpes simplex virus (HSV). The Division has therefore begun studies relevant to the future standardisation and control of these products (A. J. Garrett, with D. Warren, D. Melzack). Electrophoretic patterns of oligonucleotides derived from the endonuclease digestion of DNAs are being used to characterise strains of HSV and CMV, and possible improvements in the methods are being studied. It should then be possible to differentiate between vaccine and wild strains of virus, and to extend epidemiological work on VZV and CMV already in progress at various hospitals. An infectivity assay has been developed for VZV which will be applicable to potency assays of experimental vaccines, currently undergoing studies in various countries, including Britain.

MEASLES

As part of the ongoing MRC study on the long-term protective effects of measles vaccine, serological study of persons immunised 15 years previously,

in 1964, was continued in collaboration with the PHLS (M. Clarke). The study confirmed that a high level of protection had been maintained. A survey of antibody to measles virus in school children and young adults, also with the PHLS, is being extended. The objective is to determine whether the use of the vaccine has restricted natural infection in unvaccinated subjects, resulting in an increasing number of susceptible young adults; so far, no evidence of such a trend has been detected.

Studies on the sensitivity of methods for detecting extraneous agents in measles vaccines have led to the development of a test for avian leucoses by estimating reverse transcriptase (A. J. Garrett).

RUBELLA

Since 1977 annual surveys of rubella antibody in young adult blood donors and university students have been carried out with the PHLS (M. Clarke, with J. E. Boustred). The proportion of susceptible females is low (2–3%), reflecting vaccination at school age. In contrast, among males of the same age groups, 15–20% were seronegative. The results suggest that routine vaccination of females at school age reduces the circulation of wild rubella viruses. Susceptible males provide a potential source of infection for at-risk females in the community.

CELL SUBSTRATES

Studies have continued on detection of the potential for tumorogenicity and the presence of extraneous oncogenic viruses in cell substrates used for vaccine production (A. J. Garrett, J. P. Jacobs, with J. Hillier). The use of immunodeficient (T–B+) mice was found to be efficient for the detection of tumorogenic potential of cell cultures. Useful screening tests were developed based on the detection of the enzymes thymidine kinase and uridine kinase, induced after infection of cells with some DNA viruses, and reverse transcriptase characteristic of the RNA tumour viruses. The possibility of extending the life-span of diploid fibroblasts by the use of modified growth medium has also been examined. In some instances the life-span was increased by about 40% compared with propagation in a standard medium.

ELECTRON MICROSCOPY

This unit is available to provide a service to all NIBSC departments. It is administratively located within the Division of Viral Products, for whose work electron microscopy is essential. The unit is provided with a Phillips 201C transmission electron microscope, and acquisition of a Phillips 501B scanning electron microscope in 1980 has permitted exploration of its potential for the control of cell cultures.

Scanning electron microscopy (SEM) is still a relatively new technique and there are few proven methods for specimen preparation or accepted criteria for defining a well-preserved cell. Three features of cultured cell monolayers have been chosen which, by correlation with light and transmission electron microscopy, seem to be typical of normal cells: (1) microvilli should be uniform in shape and size and stand erect; (2) the cell membrane should be a uniform structure with no irregular holes or cracks; (3) cell junctions should show close contact with adjacent cells. Considerable progress has been made in developing techniques that can routinely secure these criteria of optimal preservation. Normal cell cultures are being studied to establish the range of features which characterise their normal surface morphology. It is anticipated that this new methodology will provide a valuable added tool for checking the health of cell cultures used for virus vaccine production.

The use of SEM for detection of infection in cell cultures is also being explored. Cultures infected with mycoplasma have been found to possess tangled filamentous structures and bleb-shaped bodies on the cell surfaces, and also an unusual single large microvillous structure. The sensitivity of these means of detecting infection in comparison with established methods is to be investigated.

The appearance of virus-infected cells under SEM is also under study and preliminary findings suggest that the technique, together with negative-staining and transmission electron microscopy, also has potential use for control purposes.

Scanning electron microscopy is also proving of value in the study of venous thrombosis (see page 32).

The Institute's *photography* service (D. Meredith) is located with the electron microscopy unit. Its limited resources are coming under increasing pressure with the growing use of photographs to record the results of, for example, electrophoretic analyses used in control testing.

Animal Services Section

The post of full-time Head of this Section has been held open, owing to financial constraints, since January 1977. It has been possible to maintain the work of the Section at a high standard only by means of a number of contributory factors. First, the Section is on two sites, the small animals at Hampstead and the primate accommodation at the MRC's National Institute for Medical Research, Mill Hill, where most of the technical staff concerned with the husbandry of the laboratory primates are MRC staff. Second is the supervision of the Animal House at Hampstead by Dr F. W. Sheffield, Head of the Bacterial Products Division. Dr Sheffield took on this task in addition to his own work when the previous Head of the Animal Services Section left early in 1977. Third is the contribution of Dr L. F. Taffs, who is responsible for much of the NIBSC scientific work at Mill Hill and who also acts as Veterinary Adviser to NIBSC. Fourth is the contribution of the technical staff involved, especially of Mr T. Cullingham, Head Technician of the Section at Hampstead. The continued absence of a full-time Head is not, however, satisfactory and the Scientific Policy Advisory Committee, following its review of the Section with the help of Dr C. Coid (CRC Northwick Park), recommended that the vacancy be filled prior to the move to Clare Hall, where all the NIBSC animal services will be located in one unit.

The Section at Hampstead is concerned entirely with the husbandry of small laboratory animals. A breeding colony of NIH mice for use in the pertussis vaccine potency test was disbanded in 1979, reliance instead being placed upon bought-in animals. Only a very limited amount of breeding is now carried out for a few strains that are not readily obtainable from accredited dealers.

Animal usage has declined over the past five years, not least owing to the development of *in vitro* techniques at NIBSC to replace *in vivo* methods. At the same time, the quality of animal husbandry has been steadily improved. Greater attention has also been given to the training of the technical staff, and efforts are being made, especially with the help of C. Chapman of the Viral Products Division, to integrate the Animal House staff into the work of the laboratory technicians.

The detailed planning of the Clare Hall Animal House has been given much attention by T. Cullingham, F. W. Sheffield and L. F. Taffs, aided by advice from Dr C. R. Coid. There are considerable complexities to the design of a modern animal house, due not only to the need to provide well-spaced accommodation with conditions of temperature control and ventilation suitable for a range of different animals, but also to ensure that infection cannot be transmitted.

Chemistry Section

The primary objective of the work of the Section is the application of physical and chemical techniques to the characterisation and analysis of biological products, with the intention of complementing and, in some cases, ultimately replacing biological assay. These techniques are applied to products recently licensed or under consideration for licensing, to materials subject to or candidates for pharmacopoeial monographs, and to the compositional and structural analysis of selected complex biological products.

In the period under review the most important technical development has been the general appreciation of the power of high performance liquid chromatography (HPLC) in the analysis of many substances of interest to the Institute. This technique, which was introduced into the Section at an early stage of its development, has been under intensive investigation and has been incorporated into NIBSC control tests for many products, for example, polypeptide hormones such as calcitonins and tetracosactrin, and antibiotics such as gentamicin and nystatin. Its application to the examination of bacterial polysaccharide vaccines is being studied, and further important developments in its use can be predicted over the next three-year period.

A significant change in emphasis of the Section's work has arisen through the introduction of bacterial polysaccharide vaccines into the United Kingdom. These vaccines cannot be tested in animal models and control greatly depends upon physical and chemical characterisation. Although there does not appear to be a major safety hazard in their use, the chemical control accepted at present does not reflect the current stage of development of polysaccharide analysis, and the application of more modern methods to their characterisation is in progress.

Control

The department has responsibility for batch control of chymopapain, all batches of which used in the UK have been examined analytically. Other substances that have been examined in conjunction with other departments include: amoxycillin, carbenicillin, cloxacillin and gentamicin (with Antibiotics); pneumococcal vaccine (with Bacterial Products); protamine (with Blood Products); and desmopressin, gonadorelin, salcatonin, seractide and tetracosactrin (with Hormones). A number of samples examined were found to be unsatisfactory, failing to comply with their specifications.

The Section is frequently involved in the investigation of *ad hoc* control problems. Those studied in the past three years include the identification of erythromycin as a contaminant of nystatin, and confirmation that the loss of potency of a batch of rubella vaccine was attributable to the anti-viral action of butylated hydroxytoluene present in excessive amounts in the rubber