PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Genetic Engineering: Possibilities and Prospects for its Application in Industrial Microbiology [and Discussion]

K. Murray, B. S. Hartley and E. G. Beveridge

Phil. Trans. R. Soc. Lond. B 1980 **290**, 369-386
doi: 10.1098/rstb.1980.0101

Phil. Trans. R. Soc. Lond. B **290**, 369–386 (1980) [369]
Printed in Great Britain

Genetic engineering: possibilities and prospects for its application in industrial microbiology

By K. Murray
Department of Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, U.K.

[Plate 1]

A wide range of techniques is now available for the construction of hybrid DNA molecules comprising components from disparate species. Transfer of segments of DNA from other organisms, and especially eukaryotes, to *Escherichia coli* permits their preparation in quantities sufficient for detailed analysis of their structure and mechanism of expression.

This information could be exploited to enhance the quantity or quality of polypeptide products from bacterial cells. Greatly increased yields of bacterial enzymes have been obtained in this way in several instances. The approaches that have been pioneered with bacteria are currently being applied to higher organisms. Much work is in progress with yeasts, in which transformation has been successfully demonstrated, with animal viruses and cells in culture and with some plant systems and offers the promise of wider applications of genetic engineering in the not too distant future.

Genetic engineering is a term subject to various interpretations, but here it concerns the manipulation of genes and, therefore, their transfer, replication and expression. A gene may be defined as the region of a chromosome responsible for some observable phenotype or characteristic of an organism. As a result of a great deal of endeavour over the past 25 years, the chemical structure of genes is well understood and the basic principles of the mechanism of their replication and expression are also clearly established. The steps involved in expression are illustrated diagrammatically in figure 1 and involve several complex interactions between proteins and nucleic acids, both DNA and RNA. Clearly a number of highly specific recognition processes between these two types of macromolecule are necessary for the correct functioning of the biological processes specified in the genes from a particular organism.

Transfer of genetic information

If genes are to be transferred effectively from one organism to another, the fidelity of the macromolecular interactions must be maintained, or it must be possible to make changes so that the appropriate macromolecular interactions can occur. The transfer of genetic material between individuals of a given species is well known and may occur by a variety of mechanisms, sexual or asexual, but there are familiar barriers to the sexual exchange of genetic information between species.

Three basic processes can be defined for the transfer of DNA between different cells (Hayes 1968). The first is transformation, a phenomenon that arises because DNA itself can be infectious, and which involves the direct uptake of DNA by the cell and was first demonstrated

in bacteria. The classical example is the change in the phenotype of a mutant bacterium after absorption of DNA from the wild type organism by cells treated in such a way as to make them 'competent' for the uptake and utilization of the DNA. When this occurs, the donor DNA is recombined into the cell chromosome, the process being recognized because the particular auxotrophic requirement is permanently relieved.

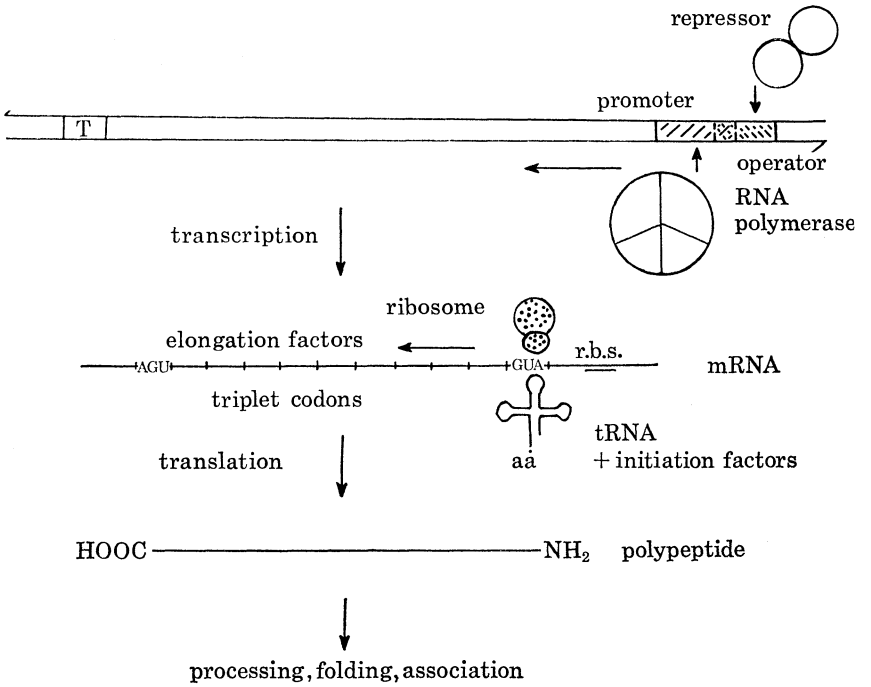

FIGURE 1. Gene expression and its regulation in bacterial systems. Many specific macromolecular interactions are involved in this process. A particular segment of the DNA molecule is transcribed by RNA polymerase to give a messenger RNA molecule. Transcription begins with the interaction of RNA polymerase at a specific site, the promoter, and the protein continues along the DNA molecule until a specific termination sequence, T, is encountered. Access of the polymerase to the promoter sequence is controlled by another protein, the repressor, which binds to a site called the operator, which is adjacent to, or overlaps, the promoter. Ribosomes bind to a particular site (r.b.s.) on the mRNA in the presence of appropriate cofactors and at the correct trinucleotide sequence (AUG). Translation is initiated by interaction with specific proteins and a tRNA molecule that has a methionine residue attached at its 3′ terminus. In the presence of elongation factors, translation of the triplet code continues until a termination codon (e.g. UGA) is encountered. The polypeptide chain then folds to give a functional protein, which in some cases may require the correct association of a number of polypeptide chains.

Transduction is a transfer process mediated by a virus (or phage) and can occur via two mechanisms. One is generalized transduction, in which any DNA within the cell concerned (including chromosomal DNA of that cell) may be encapsidated into a virus particle and then transferred to another cell after lysis of the original host cell. This is characteristic of viruses, such as coliphage P1, that package a headful of DNA in a non-specific fashion (Ikeda & Tomizawa 1965). Other viruses, such as bacteriophage λ, have the property of integrating their genome into that of their host, an essential part of lysogenization, which is normally reversible (Campbell 1971). However, the reverse of integration, i.e. excision, sometimes occurs aberrantly so that the DNA molecule excised from the lysogenic state carries with it some DNA from the host chromosome (figure 2). This DNA may then be packaged into a virus particle, which subsequently infects another host cell; this is the process of specialized transduction.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Conjugal transfer, where even the entire bacterial chromosome or bacterial plasmids may be exchanged between individual bacteria, is a sexual process and may occur between bacteria of the same or different species. When plasmids are involved, they may sometimes be integrated into the host chromosomes, so that again, by processes analogous to those of specialized transduction, genetic material may be transferred from one host organism to another.

These three natural processes vary appreciably in their range and in their specificity. The new methods involving the construction of various combinations of DNA sequences *in vitro* broaden the range of these basic mechanisms of transfer enormously.

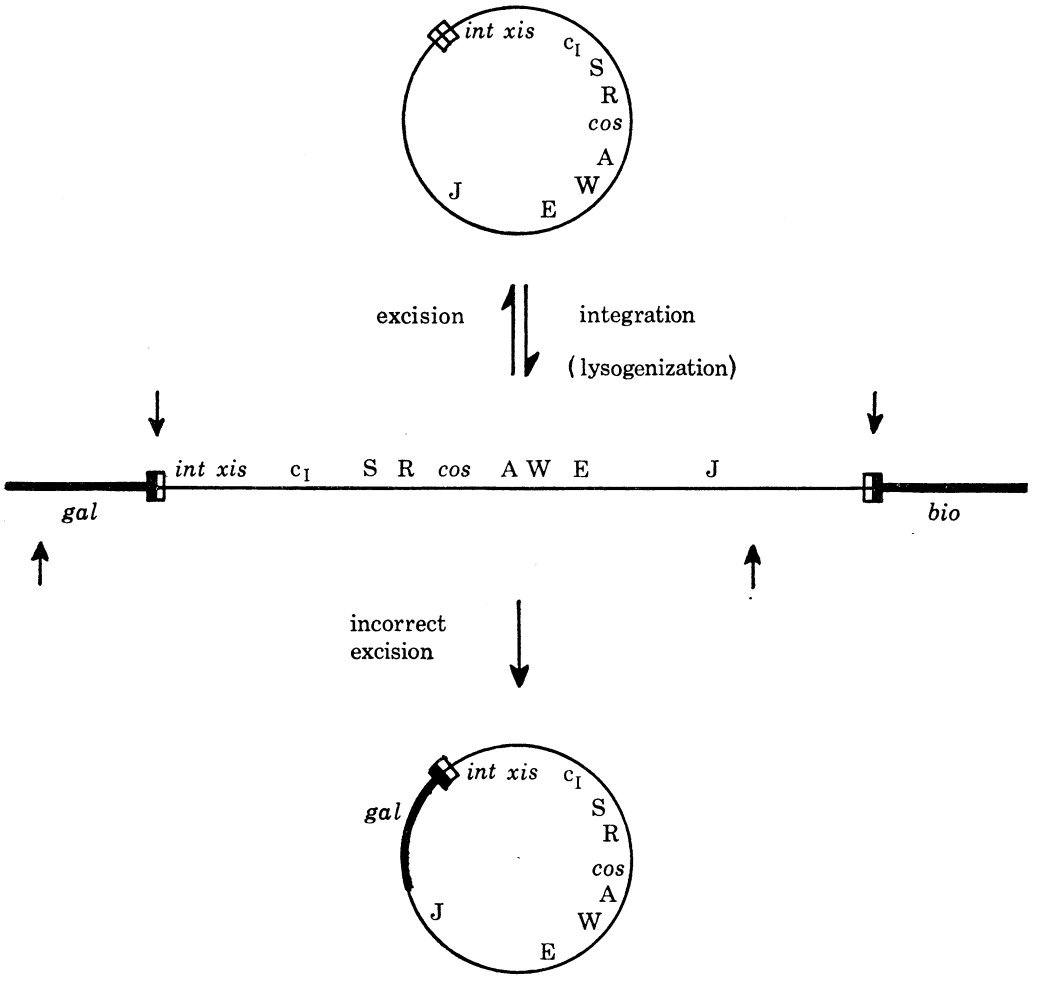

FIGURE 2. Lysogenization and the formation of specialized transducing phages. The heavy line denotes part of the host chromosome and the short vertical arrows indicate the positions of interaction of the enzymes responsible for integration or excision of the phage chromosome, the boxes showing the position (*att*) at which these reactions normally occur. The approximate position of some of the phage genes is shown (see also figure 5) and *cos* represents the position of the cohesive ends of the linear molecule formed as the phage DNA is packaged into the virion.

CONSTRUCTION OF RECOMBINANTS *IN VITRO*

The *in vitro* recombination methods centre upon the specific fragmentation of DNA molecules by restriction endonucleases. The origin of these enzymes has been discussed extensively in a number of recent reviews (Arber & Linn 1969; Roberts 1976; K. Murray 1978); for the present

TABLE 1. NUCLEOTIDE SEQUENCES RECOGNIZED BY RESTRICTION ENDONUCLEASES

bacterial source of enzyme	abbreviation	target nucleotide sequence, 5′ → 3′
Acinetobacter calcoaceticus	*Acc* I	G-T-$^{\downarrow}$A G / C T-A-C
Bacillus cereus Rf sm st	*Acc* II	C-G-C-G
Fusobacterium nucleatum D	*Bce*R	
Haemophilus influenzae 1056	*Fnu*D II	
Thermoplasma acidophilum	*Hin*1056 I	
	Tha I	
Agrobacterium tumefaciens B6806	*Atu*B I, *Atu* II	↓C-C-A/T-G-G
Arthrobacter pyridinolis	*Apy* I	
Escherichia coli/R245	*Eco*R II	
Agrobacterium tumefaciens C58	*Atu*C I	T-G-A-T-C-A
Bacillus caldolyticus	*Bcl* I	
Corynebacterium petrophilum	*Cpe* I	
Anabaena cylindrica	*Acy* I	G-R-C-G-Y-C
A. oscillarioides	*Aos* II	
A. oscillarioides	*Aos* I	T-G-C-G-C-A
Microcoleus species	*Mst* I	
Anabaena subcylindrica	*Asu* I	G-G-N-C-C
Staphylococcus aureus PS96	*Sau*96 I	
Anabaena variabilis	*Ava* I	C-Y-C-G-R-G
	Ava II	G-G-A/T-C-C
	Ava III	A-T-G-C-A-T
Arthrobacter luteus	*Alu* I	A-G-C-T
Bacillus amyloliquefaciens H, or F, or K, or N	*Bam*H I	G-G-A-T-C-C
Bacillus stearothermophilus	*Bst* I	
Bacillus brevis S	*Bbv*S, *Bbv* I	G-C-A/T-G-C
B. cereus	*Bce*l70	C-T-G-C-A-G
B. subtilis l247	*Bsu*l247	
Providencia stuartii l64	*Pst* I	
Streptomyces albus Pl	*Sal*P I	
Xanthomonas malvacearum	*Xma* II	
X. oryzae	*Xor* I	
Bacillus globigii	*Bgl* II	A-G-A-T-C-T
B. sphaericus R	*Bsp*R I	G-G-C-C
B. subtilis R (x5)	*Bsu*R	
Brevibacterium luteum	*Blu* II	
Fusobacterium nucleatum D	*Fnu*D I	
Haemophilus aegyptius	*Hae* III	
H. haemoglobinophilus	*Hhg* I	
Moraxella nonliquefaciens	*Mnn* II	
Neisseria gonorrhoea	*Ngo* II	
Providencia alcalifaciens	*Pal* I	
Streptococcus faecalis	*Sfa* I	
Bordatella bronchiseptica	*Bbr* I	A-A-G-C-T-T
Corynebacterium humiferum	*Chu* I	
Haemophilus influenzae b, or d	*Hin*b III, *Hin*d III	
H. suis	*Hsu* I	

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

bacterial source of enzyme	abbreviation	target nucleotide sequence, 5′ → 3′
Brevibacterium albidum	*Bal* I	T-G-G-C-C-A
B. luteum	*Blu* I	C-T-C-G-A-G
Streptomyces albus pathociclicus	*Spa* I	
S. exfoliatus	*Sex* I	
S. goshikiensis	*Sgo* I	
S. lavendulae	*Sla* I	
S. luteoreticuli	*Slu* I	
Xanthomonas holcicola	*Xho* I	
X. papavericola	*Xpa* I	
Caryophanon latum L	*Cla* I	A-T-C-G-A-T
Corynebacterium humiferum	*Chu* II	G-T-Y-R-A-C
Haemophilus influenzae c, or d	*Hinc* II, *Hind* II	
Moraxella nonliquefaciens	*Mnn* I	
Diplococcus pneumoniae	*Dpn* I, *Dpn* II	↓G-A-T-C
Fusobacterium nucleatum A, or C, or E	*Fnu*A II, *Fnu*C I, *Fnu*E I	
Moraxella bovis	*Mbo* I	
M. osleonsis	*Mos* I	
Staphylococcus aureus 3A	*Sau*3A	
Enterobacter cloacae	*Eca* I	G-G-T-N-A-C-C
Escherichia coli B	*Eco*B	T-G-A-$(N)_8$-T-G-C-T
E. coli RY13	*Eco*R I	G-A-A-T-T-C
	*Eco*R I′	R-R-A-T-Y-Y
	*Eco*R I*	↓A-A-T-T
Fusobacterium nucleatum A	*Fnu*A I	G-A-N-T-C
Haemophilus haemolyticus	*Hha* II	
H. influenzae f	*Hinf* I	
Fusobacterium nucleatum D	*Fnu*D III	G-C-G-C
Haemophilus haemolyhicus	*Hha* I	
Moraxella nonliquefaciens	*Mnn* IV	
Haemophilus aegyptius	*Hae* I	$\begin{matrix}A\\T\end{matrix}$-G-G-C-C-$\begin{matrix}T\\A\end{matrix}$
H. influenzae H	*Hae* II	R-G-C-G-C-Y
Neisseria gonorrhoea	*Hin*H I	
	Ngo I	
Haemophilus aphrophilus	*Hap* II	C-C-G-G
H. parainfluenzae	*Hpa* II	
Moraxella nonliquefaciens	*Mno* I	
Moraxella species	*Msp* I	
Haemophilus gallinarum	*Hga* I	G-A-C-G-C 5or10bp
H. parahaemolyticus	*Hph* I	G-G-T-G-A 8or7bp
H. parainfluenzae	*Hpa* I	G-T-T-A-A-C
Herpetosiphon giganteus	*Hgi*A I	G-$\begin{matrix}T\\A\end{matrix}$-G-C-$\begin{matrix}T\\A\end{matrix}$-C
Klebsiella pneumoniae	*Kpn* I	G-G-T-A-C-C
Moraxella bovis	*Mbo* II	G-A-A-G-A 8or7bp
M. nonliquefaciens	*Mnl* I	C-C-T-C 5or10bp

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE 1 (*cont.*)

bacterial source of enzyme	abbreviation	target nucleotide sequence, 5′ → 3′
Proteus vulgaris	*Pvu* I	C-G-A-T-C-G
Rhodopseudomonas sphaeroides	*Rsh* I	
Xanthomonas nigromaculans	*Xni* I	
X. oryzae	*Xor* II	
Proteus vulgaris	*Pvu* II	C-A-↓G-C-T-G
Serratia marcescens	*Sma* I	C-C-↓C-G-G-G
Streptomyces faecalis ND574	*Sfa*N I	G-A-T-G-C
Streptomyces achromogenes	*Sac* I	G-A-G-C-T-↓C
S. stanford	*Set* I	
S. achromogenes	*Sac* II	C-C-G-C-G-↓G
S. stanford	*Set* II	
Thermopolyspora glauca	*Tgl* I	
Streptomyces albus G	*Sal* I	G-T-C-G-A-↓C
Xanthomonas amaranthicola	*Xam* I	
Thermus aquaticus	*Taq* I	T-C-G-↓A
Xanthomonas badrii	*Xba* I	T-C-T-A-G-↓A
X. malvacearum	*Xma* I	C-C-C-G-G-↓G

All of the enzymes break double stranded DNA, but the sequence is shown for only one strand. The arrow shows the position of the bonds broken; and, in those cases analysed, breakage occurs to leave a 5′-phosphate and 3′-hydroxyl group. Most of the sequences, and positions of bonds broken, are rotationally symmetrical. R and Y are the standard abbreviations for purine and pyrimidine, respectively. The abbreviated description of the enzymes is that proposed by Smith & Nathans (1973). The table is compiled largely from the lists prepared by Roberts (1976, 1980), which contain literature references for all the enzymes and give additional characteristics of the enzymes and also include several more enzymes for which the recognition target sequences are not yet known. The enzymes *Acy* I, *Aos* I and *Aos* II are described by de Waard *et al.* (1978, 1979). Enzymes from the bacteria inset recognize the same target sequence as that for the preceding entry.

purposes they may be regarded as enzymes that degrade DNA from any source into a series of discrete fragments, the size of which is determined by the frequency with which the nucleotide sequence recognized by the enzyme occurs in the DNA. A large number of these enzymes with different nucleotide recognition specificities is now known and the size of the recognition sequence, as well as its base sequence, varies (table 1). The frequency with which any particular nucleotide sequence would occur by chance in a polynucleotide of uniform base composition is 4^{-n}, where n is the number of bases comprising that sequence. Thus a given tetranucleotide would occur once in 256 base pairs and a specific hexanucleotide once in 4096 base pairs. DNA molecules are not random polymers, but these values indicate the approximate size of fragments that one might expect by chance and in practice there is a very broad spread around these values. The important point is that the sizes of DNA fragments produced are such that many would encompass a gene or, in some cases, several genes, for each amino acid of a protein is defined by a sequence of three nucleotides.

DNA from any organism may be degraded into a series of fragments. Frequently the mixture of fragments will be very complex, but there are several methods available for fractionation of the digests and, in simpler cases, such as viral DNA, for recovery of individual fragments (figure 3). In many instances the position at which the enzyme breaks the recognition sequence

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(table 1) gives fragments with cohesive ends, which permit the fragments to reassociate with each other by normal Watson–Crick base pairing; the action of DNA ligase then joins the fragments together again covalently. Recent developments in nucleic acid biochemistry have provided a range of methods for joining DNA fragments together regardless of the nature of their ends (K. Murray 1978). The newly constructed molecules may be introduced by transformation into microorganisms where they would not normally occur.

TABLE 2. THE *DESIDERATA* OF A DNA MOLECULE THAT IS TO SERVE AS A VECTOR FOR DNA FRAGMENTS IN *IN VITRO* RECOMBINATION EXPERIMENTS

1. The molecule must be capable of autonomous replication in an appropriate host cell.
2. The site for insertion of a DNA fragment must be such that the insertion does not destroy an *essential* function.
3. The vector must carry a means for selection of transformed cells, such as a drug resistance determinant, the ability to confer immunity (for example to a colicin or to a phage), or the production of a phage plaque in a lawn of bacterial cells.
4. A means for distinguishing, or preferably selection of, recombinant DNA molecules from the parent vector DNA.

TRANSFORMATION OF BACTERIA, AND THE RECOGNITION OF CELLS CARRYING RECOMBINANTS

Bacterial transformation is recognized as a phenotypic change consequent upon the uptake of DNA. The process is usually rather inefficient and can only be observed with an appropriate selective system; DNA fragments or molecules may thus be absorbed into cells, but the process passes unobserved. However, many DNA molecules, such as bacterial plasmids or viruses, the presence of which can be demonstrated readily, can be taken up by bacterial cells and will replicate autonomously in these cells. If DNA fragments produced by restriction enzyme digestion are attached to such molecules, these additional sequences will be replicated along with the plasmid (or virus), which is therefore termed a vector (or receptor) for the introduction of the DNA fragments into bacterial cells, the transformed cells being recognized by virtue of a selectable marker carried by the vector. This provides the means of cloning genes, or fragments of them, from any organism in bacteria, because, if transformation is carried out at a low DNA concentration, there is a high probability that any single transformant will have acquired only one DNA molecule and, therefore, a single fragment of DNA from the complex organism under study.

The necessary characteristics of a plasmid, or phage, vector for this type of experiment are summarized in table 2. Several methods are available for detection of specific recombinants. The simplest, where applicable, relies upon the complementation of an auxotrophic host strain (Borck *et al.* 1976). Where transformation by the recombinant plasmid or phage leads to the production of particular enzymes or their metabolic products, it is often possible to detect these by a simple colour test. For example, introduction (by transformation) of recombinants that contain the *lac* gene of *Escherichia coli* permits the synthesis of β-galactosidase by cells that otherwise cannot make the enzyme because of a defect (an *amber* mutation, for example) in this particular gene. There are convenient and sensitive colour tests, such as the use of lactose MacConkey agar plates (red colonies) or 5-bromo-2-chloroindolyl-β-D-galactoside (blue colonies or phage plaques), by which cells producing β-galactosidase can

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

readily be distinguished from those that are not (Davies & Jacob 1968). This type of experiment clearly has wide application. Cells that carry recombinant phage as a prophage can also be detected in an equivalent manner. Strains unable to hydrolyse glutamine, for example, when lysogenized with a phage carrying the glutaminase gene may be detected readily on appropriate indicator plates, by the pH change accompanying the release of ammonia.

Recombinant phage made by the *in vitro* methods can often prove useful themselves as vectors, because restriction of the phage DNA enables the inserted fragment to be replaced by a different DNA fragment. If the fragment being replaced contains a readily detectable or selectable marker, this gives a very convenient way of distinguishing between the vector and new recombinant phages. Lambdoid phage that contain the *lac* gene or a suppressor tRNA gene (derived from *E. coli*) are examples of such replacement vectors, for substitution of this gene by another DNA fragment will remove the ability of the phage to complement a *lac*

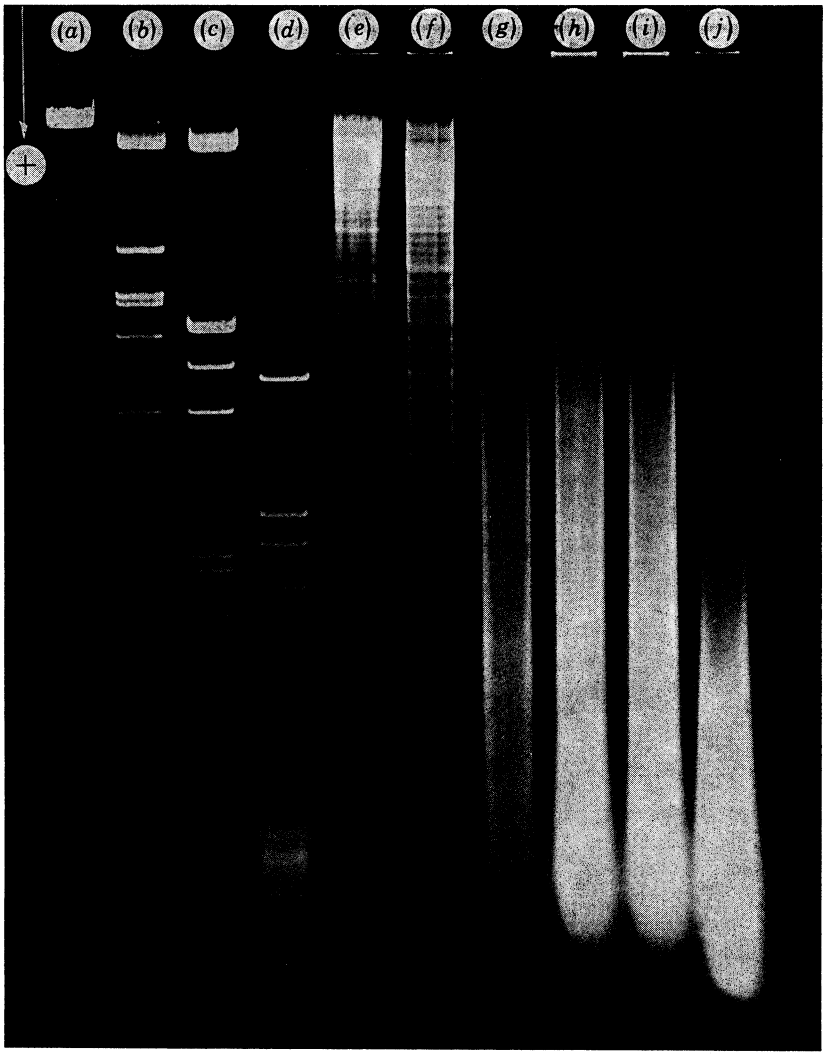

FIGURE 3. For description see opposite.

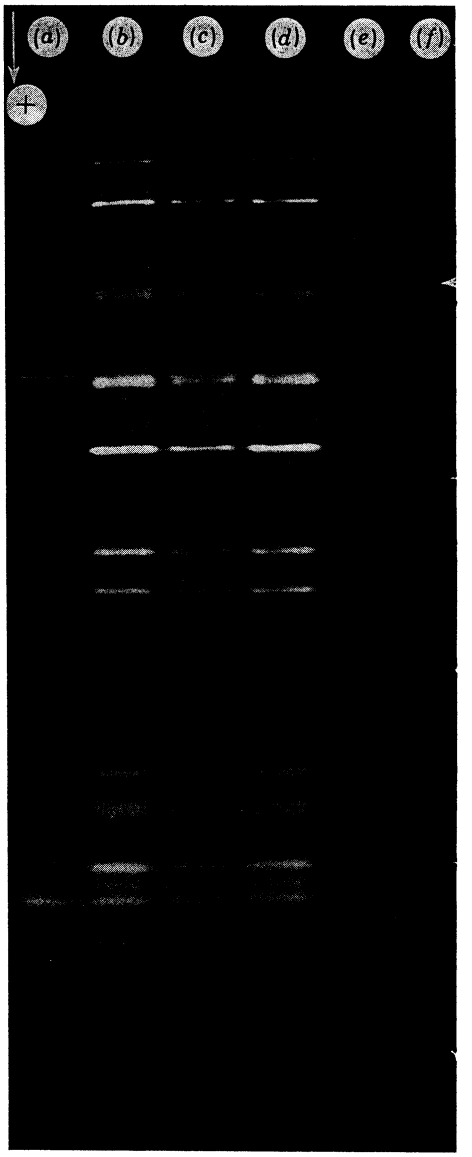

FIGURE 4. For description see opposite.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

amber host strain, and so these phage will give colourless plaques on appropriate indicator plates.

A wide range of phage and plasmid vectors is now available. A description of the various types and their characteristics is given in reviews by N. E. Murray (1978) and by Sutcliffe & Asubel (1978).

Frequently, genes incorporated into the recombinant will not be manifest by their expression, but they may be detectable by nucleic acid hybridization methods, which can be applied directly to bacterial cells (Grunstein & Hogness 1975) or phage plaques (Benton & Davis 1977). Where a suitable radioactive nucleic acid, such as a purified mRNA or cDNA is available, this method provides an extremely powerful means for identification of a particular recombinant in a large population. In conjunction with the *in vitro* packaging of phage genomes (Hohn & Murray 1977; Sternberg *et al.* 1977), the method permits very large numbers of recombinants to be screened with high efficiency and it is possible to detect one sequence in a million. An analogy in chemistry might be a situation where one is confronted with a mixture of all the reagents in the laboratory and one has the means to locate a rare contaminant from one of these components in the mixture, to isolate it and then to purify it and produce it in sufficient quantity to work out the entire chemistry of a new element.

Other procedures for detection and characterization of recombinants include immunological methods employing fluorescent or radioactively labelled antibodies (Sanzey *et al.* 1976; Skalka & Shapiro 1977; Broome & Gilbert 1978), and electrophoresis of cell extracts. The latter may be used for direct analysis of the plasmids by measurement of mobilities in agarose gels, which

FIGURE 3. The separation of DNA fragments in restriction enzyme digests by electrophoresis in a 10 g/l agarose gel. The results show the effects of the size of the target sequence for the restriction enzyme and the complexity of the DNA preparation upon the size and number of fragments obtained. Electrophoretic mobility of a DNA fragment is inversely proportional to the logarithm of its relative molecular mass. In the first four tracks are samples of phage λ DNA, a relatively simple molecule with a molecular mass of 3.2×10^7: (*a*) before digestion and (*b*) after digestion with R.*Eco*R I; (*c*) a mixture of R.*Eco*R I and R.*Hin*d III (both of which recognize hexanucleotide sequences); and (*d*) R.*Hae* III (which recognizes a tetranucleotide sequence). The next three tracks contain corresponding digests of *E. coli* DNA, which is about a hundred times the size of phage λ DNA; resolution of individual bands is still possible, at least partially, at the analytical level. The last three tracks contain digests of human liver DNA with the same enzymes and here the complexity of the DNA is so great that resolution of individual fragments is lost, although bands arising from the breakage of highly reiterated sequences may often be discerned. There are several methods for the recovery of DNA from agarose gels, some based upon dissolution of the gel in a chaotropic agent such as a saturated aqueous solution of potassium iodide. In this experiment, 0.5–3.0 μg DNA was digested and after electrophoresis the gel was stained with ethidium bromide and photographed under ultraviolet light (Sharp *et al.* 1973).

FIGURE 4. Analysis of labelled polypeptides in extracts of phage-infected cells by electrophoresis in polyacrylamide gels. The host cells were irradiated with ultraviolet light to minimize expression of host genes and then infected with the phages. [^{35}S]Methionine was added within 2 min of adsorption of the phage and then diluted with a large excess of non-radioactive methionine after 5 min so that only proteins synthesized within this period were labelled. The cells were dissolved in a buffer containing sodium dodecyl sulphate (SDS) for electrophoresis through a 100–200 g/l gradient polyacrylamide gel, which was then dried and radioautographed. The experiment identifies the band attributable to T4 DNA ligase and shows the production of this polypeptide in cells infected with a phage derivative carrying the T4 DNA ligase gene (Wilson & Murray 1979). The samples in the various tracks are: (*a*) uninfected host cells; (*b*) cells infected with T4$^+$; (*c*) cells infected with T4g30 *am* (i.e. an *amber* mutation in the DNA ligase gene); (*d*) cells infected with T4g41 *am* (the gene 41 product has a mobility very close to that of DNA ligase; comparison of tracks (*b*), (*c*), and (*d*) shows the band (indicated by the horizontal arrow) attributable to DNA ligase; (*e*) cells infected with a λT4 *lig* phage, where expression of the T4 gene is under control of the early λ promoter P_L; (*f*) cells infected with a similar λT4 derivative, where the T4 DNA fragment is inserted in the opposite orientation.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

gives their relative molecular masses (r.m.m.) (Sharp *et al.* 1973), or, alternatively, and usually after labelling with radioactive isotopes, for characterization of polypeptides synthesized by cells carrying the recombinant plasmids or infected with phage (Jaskunas *et al.* 1975; Murray *et al.* 1979). These methods have now been widely used and have the merit that, if the product is an enzyme, it need not be biologically active for its detection. An example of the electrophoretic analysis is shown in figure 4.

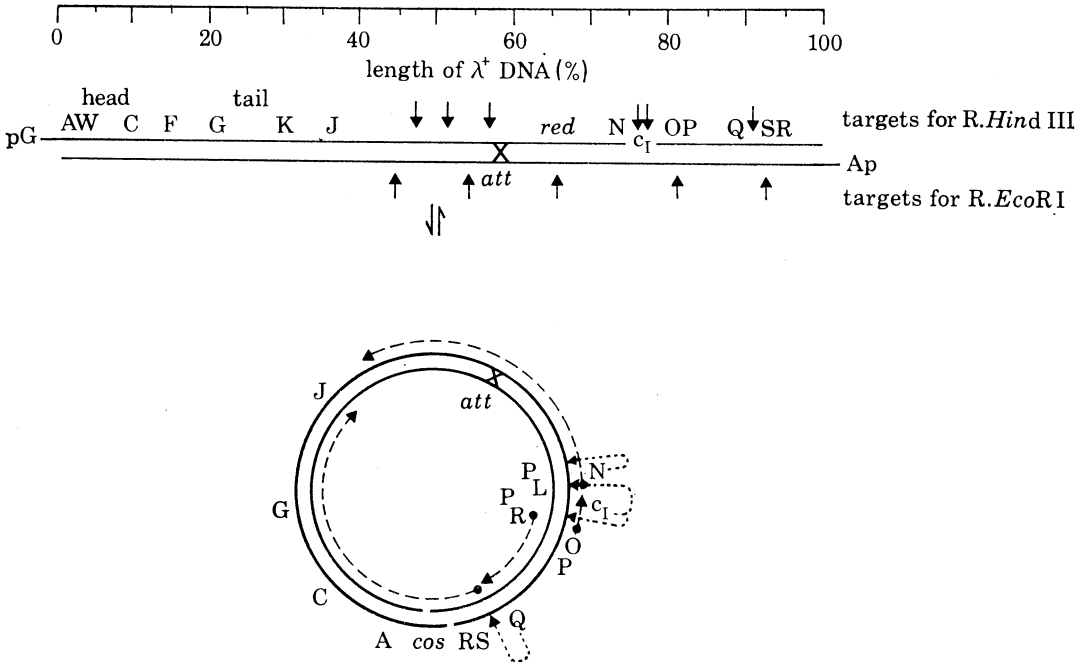

FIGURE 5. A simplified version of the map of the bacteriophage λ genome, showing some of the more important control systems. The mature phage contains a single, linear duplex DNA molecule with a relative molecular mass of 3.2×10^7. At the 5′ ends of the DNA are single-stranded projections of 12 bases with complementary sequences. Upon injection into the host cell, the DNA circularizes by base pairing of these cohesive ends. Chromosomes of lambdoid phages are normally drawn in the linear form; here genes are located at the positions corresponding to the percentage of the length of the wild-type phage DNA. Genes on the left of the linear map code for head and tail proteins of the phage. Much of the central region is inessential and can be deleted without seriously impairing phage growth. *Red* represents the phage recombination system, O and P are concerned with replication of the phage DNA, and S and R code for proteins that lyse the host cell when the phage products have been assembled into infectious particles. The c_I gene codes for a repressor protein that interacts at the sites shown by the dotted arrows in the lower part of the figure to prevent expression of the phage genes. Removal of the repressor permits expression in both directions from P_L and P_R, as shown by the broken arrows. N and Q are positive regulatory genes, the products of which interact at the positions shown by dotted arrows. Q is necessary for the expression of genes S and R and genes to the left of these, as indicated by the long broken arrow inside the circle. Thus, after circularization of the chromosome, gene Q activates the expression of genes A, C, E etc. (i.e. those on the left of the linear map) as well as genes R and S. Also shown in the figure is the attachment site by which the phage chromosome may be inserted into its host chromosome (where it may be stably replicated along with the host), and the positions of targets for the restriction enzymes R.*Hin*d III and R.*Eco*R I in the wild-type chromosome. Some of these targets must be removed by deletion or mutation in order to make phage derivatives that can be used as receptors. (Reproduced, with permission, from Murray 1976.)

GENE EXPRESSION AND ITS AMPLIFICATION

A number of the methods for detection of foreign genes introduced into bacteria depend upon expression of these genes in their new environment. Where this occurs, it is often possible to amplify the level of expression and derivatives of phage lambda are particularly useful for

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

this purpose. Basically, two parameters influence the level of expression; the number of gene copies and the efficiency of their transcription and translation. Phage lambda has been studied intensively and the position of many of the virus genes as well as their control elements is known with precision (Hershey 1971); some of the more important of these are shown in figure 5. The amplification of T4 DNA ligase, an enzyme that is particularly useful in the recombinant DNA methodology, provides an example of the way in which the basic genetics of lambda may be exploited. DNA from phage T4 was digested with restriction enzymes, the digestion products inserted into lambda vectors, and recombinants recovered by transfection of a suitable host strain (Wilson & Murray 1979). The population of recombinants was then screened for phage that complemented a ligase-deficient strain of *E. coli* and phage that on this basis expressed the ligase gene were purified and use to study the level of the enzyme in infected host cells (Murray *et al.* 1979). In figure 6 the amount of ligase obtained from such cells is compared with that from an equivalent quantity of *E. coli* B infected with a T4 strain.

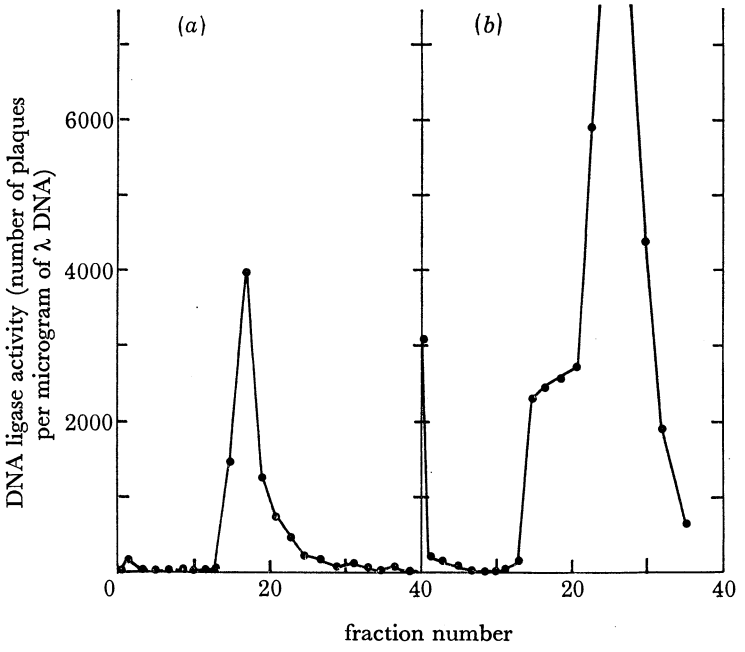

FIGURE 6. Comparison of the yield of T4 DNA ligase from *E. coli* infected with (*a*) T4 and (*b*) a λ–T4 hybrid. Similar masses of the two cell pastes were extracted and the figure shows chromatographic fractionations on DEAE-Sephadex A50. The assay measures the restoration of infectivity (in a transfection assay) of a phage λ DNA cleaved into two fragments by a restriction enzyme (Murray *et al.* 1979).

Preparative experiments are best performed with thermo-inducible lysogens of the recombinant phage and various aspects of lambda can be exploited to optimize expression of the inserted gene and isolation of its product. Use of the efficient lambda promoters increases the level of transcription of the incorporated genes and an amber mutation in gene *S* prevents lysis of the host so that gene products are contained within the cells. (This is useful for laboratory scale preparations, but would not be chosen for large scale operations.) For T4 DNA ligase, an important additional factor is the genetic purification afforded by transfer of the T4 genes to lambda, whereby the ligase gene is isolated from other T4 genes, many of which code for potent nucleases that would interfere with the subsequent purification of the enzyme. More

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

detailed manipulation of the recombinant phage gave further amplification of the enzyme on induction of appropriate lysogens. The transcription best relied upon towards the right from the late λ promotor P'_R under the positive control of gene Q in a phage with *amber* mutations in gene *S* (necessary for cell lysis) and gene *E*, the product of which is required for production of phage heads. Induction of the prophage permits replication of the phage DNA to give many gene copies, which, in the absence of the *E* gene product, cannot be encapsidated and so remain available for transcription to continue for prolonged periods, and translation products of these transcripts accumulate within the cells since lysis is blocked. In this way, cells have been obtained with a ligase content of about 2 % of the soluble cellular protein (Murray *et al.* 1979). DNA ligase from *E. coli* or from *E. coli* infected with phage T4 has been widely used for the covalent linkage of double-stranded DNA fragments that have been joined together by the cohesion of short single-stranded projections with complementary sequences. The two enzymes are quite different and have different cofactor requirements, and the T4 enzyme differs from that of *E. coli* in that it is capable of covalently joining DNA fragments that do not have projecting terminal nucleotides (Sgaramella *et al.* 1970), It is claimed that this so-called 'blunt-ended' ligation reaction is stimulated by the T4 RNA ligase (Sugino *et al.* 1977), but it is of interest to note that the T4 DNA ligase prepared from the λ–T4 hybrid phage, which does not contain the RNA ligase gene, is capable of ligating together small, blunt-ended DNA fragments, so the RNA ligase cannot be essential for this reaction (Murray *et al.* 1979).

Genes for several other bacterial enzymes have been manipulated in similar ways, giving amplification often of a few hundred fold of the normal level. These include DNA polymerase (Kelley *et al.* 1977; Murray & Kelley 1979) and DNA ligase from *E. coli* (Cameron *et al.* 1975), as well as some restriction and modification enzymes. In some instances, however, problems arise due to the lethal effects of high levels of a particular gene product. In such instances, it may be useful to work with cells lysogenized with a phage that can be induced at the appropriate stage of the growth cycle so that the recombinant is propagated as a single gene copy per bacterial chromosome and then induced to give many functional gene copies at a stage when the cells can be sacrificed.

Expression of eukaryotic genes in bacteria

The expression of eukaryotic genes in bacterial cells is less straightforward than was at first imagined. Not only is there the problem of incompatible sequences for recognition by the appropriate protein-synthesizing machinery, but the structure of at least some eukaryotic genes is now known to differ fundamentally from that of bacterial genes in that they contain interruptions (so-called introns) of considerable stretches of nucleotide sequence within the coding region (Breathnach *et al.* 1978; Chow *et al.* 1977; Jeffreys & Flavell 1977; Tilghman *et al.* 1978). Transcription of this gives a faithful RNA copy, but this RNA molecule is then folded so that some enzyme removes part of the sequence and splices together the other pieces to give a true messenger RNA molecule (figure 7). Thus, when a genomic sequence is cloned and transcribed, the bacterium is presented with a novel situation in the form of an RNA molecule that cannot be processed. The hiatus can be circumvented by using reverse transcriptase to make a DNA copy of a mature messenger RNA. This DNA (cDNA) may then be introduced into an appropriate phage or plasmid vector for propagation in bacteria, the choice of the vector system depending upon the objective of the particular experiment.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

An interesting approach has been used by Gilbert and his colleagues with the plasmid pBR322, which contains a single target for the restriction enzyme *Pst* located within the gene that determines resistance to ampicillin. Introduction of a fragment of DNA at this position inactivates the ampicillin resistance gene, but the plasmid also contains a gene that determines resistance to tetracycline, so that transformed cells may still be selected readily. This system was used to insert cDNA for rat insulin into the plasmid so that its transcript would be fused to that of the penicillinase gene (figure 8). The insertion was made by the so-called 3′-tailing

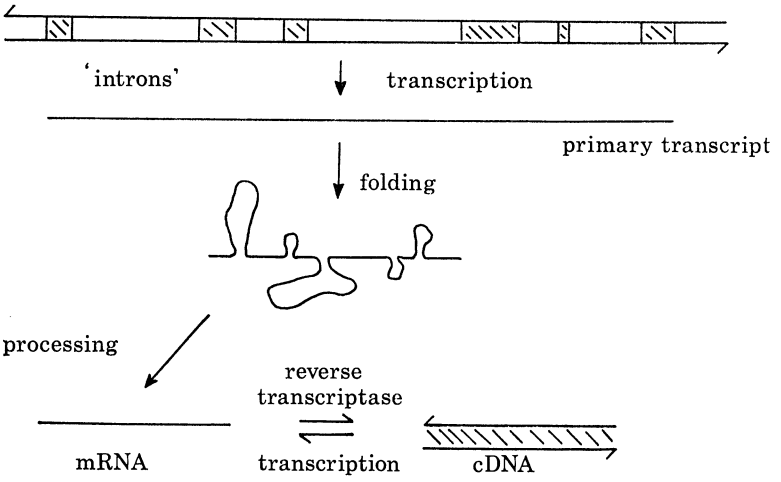

FIGURE 7. A diagram illustrating the organization and expression of some eukaryotic genes. The sequence coding for a polypeptide, represented by the hatched area, is interrupted by additional non-coding sequences called introns, which may be quite long. The primary transcript must be processed (spliced) to give a mature mRNA molecule, which is translated in the normal way (figure 1). The action of reverse transcriptase upon a mature mRNA gives a DNA molecule containing uninterrupted coding sequences analogous to gene sequences in bacteria.

method (Jackson *et al.* 1972), in which polynucleotide terminal transferase is used to extend the 3′ terminal residues of the DNA fragments to be joined with homo-oligomeric nucleotide sequences, the nucleotide being chosen so that 3′ extensions of one component are complementary to those of the other. The two DNA components are then joined by cohesion of the complementary oligomeric sequences and the preparations may be used directly for transformation of competent host cells. The products of the terminal transferase reaction comprise populations of molecules with differing lengths of the 3′ extension, and therefore the recombinants recovered by transformation will include a range of molecules that differ in the number of A-T or G-C base pairs (depending upon the nucleotides selected for the terminal transferase reactions) by which the two DNA components have been joined. Thus there will be a one in six probability that a transcript initiated at the penicillinase promoter and continuing through the inserted DNA sequence will contain the information from the inserted DNA in the correct orientation and reading frame, or phase, for translation to give a polypeptide consisting of the N-terminal segment of penicillinase linked by a few glycine residues to the gene product under study. An added advantage of this procedure is that the penicillinase has a hydrophobic N-terminal sequence (leader sequence), which allows the enzyme to be transported through the membrane to the cell surface. This leader sequence should therefore transfer the fused polypeptide to the surface of the cell, permitting its detection by immunological reagents for either component of the polypeptide.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

A solid phase radioimmunoassay has been developed for this purpose for the rapid screening of cells producing such products, and utilizes a plastic sheet coated with antibodies to the protein under study (Broome & Gilbert 1978; Erlich *et al.* 1978).

Cells producing the particular protein (i.e. the corresponding antigen) should interact specifically when pressed against the coated surface. Areas of this surface to which the antigen has thus become attached will then be able to absorb radioactively labelled antibody molecules, whereas the labelled antibodies will not stick to the precoated layer of the same antibodies.

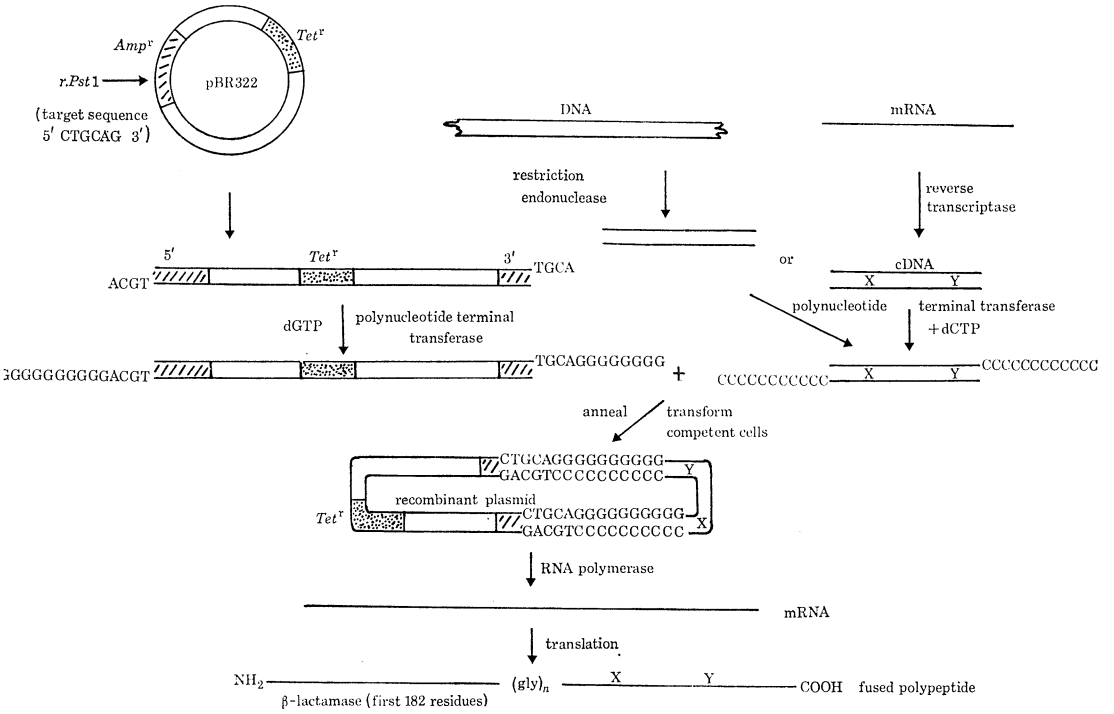

FIGURE 8. The insertion of DNA fragments into the β-lactamase gene of the *E. coli* plasmid pBR322 for expression of the inserted genes as polypeptides fused to β-lactamase. The plasmid contains a single target for the restriction enzyme R. *Pst* I, which is located within the gene that determines resistance to ampicillin (*Amp*^{r}) and through which it is converted into a linear molecule. Oligo(dG) sequences are then synthesized at the 3′ termini by the action of polynucleotide terminal transferase. DNA fragments to be joined to these molecules are prepared either by digestion of a DNA preparation with restriction enzymes (or by shearing) or by the action of reverse transcriptase upon an RNA preparation, and oligo(dC) sequences are synthesized at their 3′ termini as before. Recombinant plasmids formed by annealing the DNA molecules with complementary 3′ single-stranded projections effectively transform competent cells, the necessary exonucleolytic, synthetic and ligation reactions being completed *in vivo* to give the covalently linked recombinant. (Note that the recognition target for R. *Pst* I is regenerated at the junctions of the two components.) Transcription from the β-lactamase promoter gives an mRNA that is translated to a polypeptide comprising the first 182 residues of β-lactamase (penicillinase) and, when the number of connecting G residues is such as to maintain the correct translational phase, this is linked by a few glycine residues to the polypeptide sequence from the cloned gene or DNA fragment of interest. Although this does not give a functional penicillinase, the gene for tetracyclin resistance (*Tet*^{r}) remains intact and provides a means for selecting transformants.

In other words, the antigen produced by the bacteria provides a means of attaching the radioactively labelled antibody, which can then be detected radioautographically (figure 9, plate 1). This gives a very powerful method for the detection of an antigen produced in a bacterial cell and was used successfully to demonstrate the bacterial synthesis of both rat and human insulins (Villa-Komaroff *et al.* 1978; W. Gilbert, personal communication). The yield of insulin (or proinsulin) was not high, probably because the new polypeptide was degraded rapidly within

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

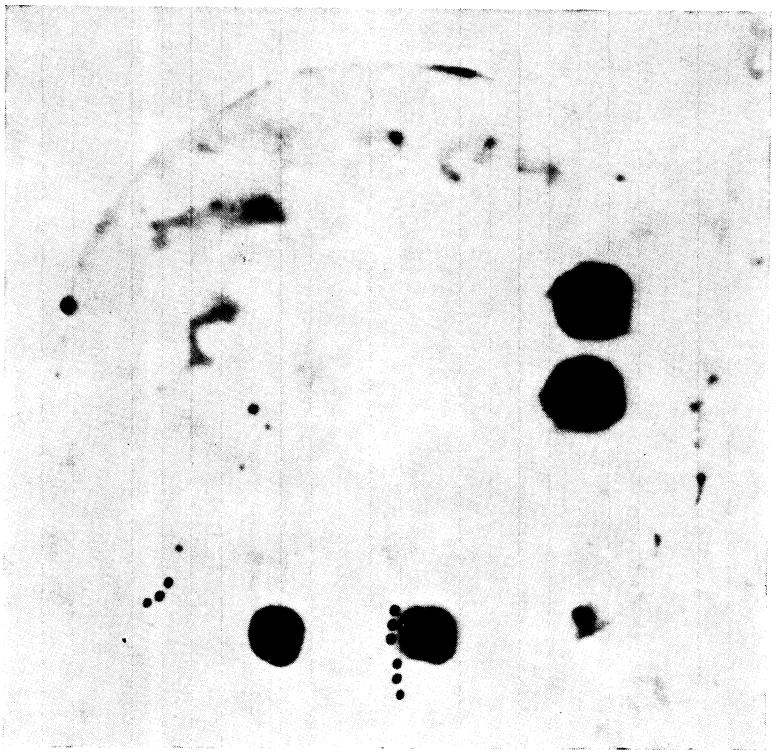

FIGURE 9. The radioimmunoassay for detection of bacterial colonies producing an antigenic polypeptide. The example (from Burrell *et al.* (1979)) shows that 4 out of the 52 colonies imprinted upon the plastic disk coated with anti-HBc gave positive reactions when the disk was subsequently treated with ^{125}I-labelled HBc antibodies.

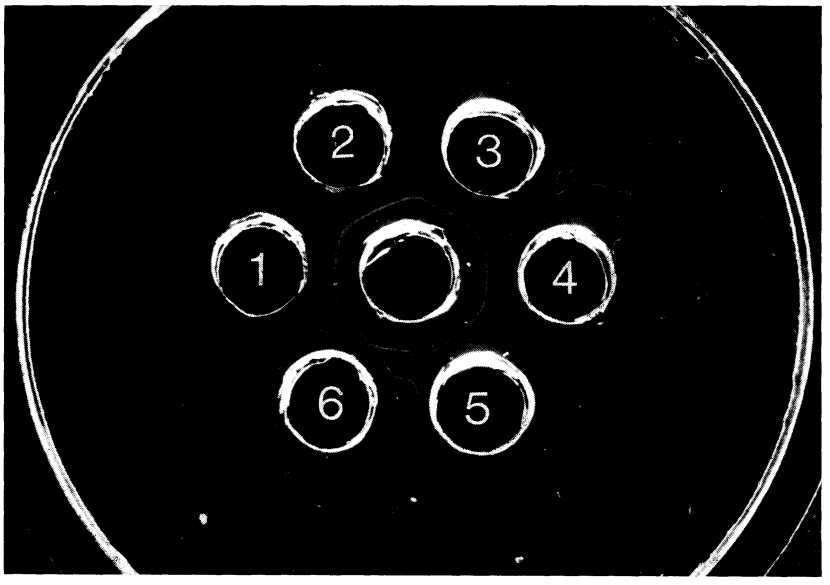

FIGURE 11. Comparison, by the immunodiffusion method, of human antibodies to HBcAg with those from rabbits injected with HBcAg made in *E. coli*. The centre well of the gel (9 g/l agarose in 0.01 M Tris–HCl pH 7.2, 0.001 M EDTA, 0.1 M-NaCl) contained partially purified HBcAg derived from human liver. Wells 1 and 4 contained human anti-HBc at a dilution of 1 in 30; wells 2 and 3 contained undiluted serum from rabbits injected with the unfractionated extract of bacteria carrying clone pHBV139; wells 5 and 6 contained undiluted rabbit serum raised against HBcAg from the same bacterial extract, but fractionated on a column of Sephadex G50 (Pasek *et al.* 1979).

(*Facing p.* 382)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

the bacterial cell. This method has also been used to demonstrate the production in *E. coli* of growth hormones (Seeburg *et al.* 1978) and, as will be described later, human viral antigens. Processing of the fused polypeptide to give the desired gene product alone is a more complex problem.

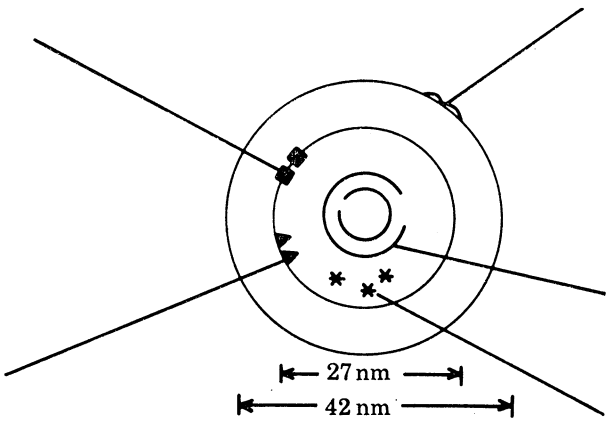

FIGURE 10. Hepatitis B virus; its components and a diagrammatic representation of its structure. The intact virion has a diameter of 42 nm, often referred to as a Dane particle, from which the smaller core particle is derived. The surface antigen often occurs in a highly associated form visible under the electron microscope as cylindrical or rod-like structures. This figure was kindly provided by Dr Patricia MacKay.

EUKARYOTIC VECTORS

Eukaryotic cloning systems have also been developed and offer an alternative route to solution of problems in gene expression related to the structure of the eukaryotic genome. One is the transformation of yeast cells, which was achieved by Hinnen *et al.* (1978), who used a hybrid ColE1 plasmid containing yeast DNA to transform a *leu*$^-$ auxotroph of yeast to *leu*$^+$ and showed that both yeast and ColE1 DNA sequences from the transforming plasmid were integrated into the yeast genome. The transformation frequency was very low, but much higher efficiencies have been achieved through the use of a vector that consists of the *E. coli* plasmid pMB9 (a derivative of ColE1) and a small circular DNA molecule isolated from yeast (usually referred to as the yeast 2 μm circular DNA). This hybrid plasmid can replicate in either yeast or *E. coli* and, using again the yeast *leu*2 allele, Beggs (1978) found that recombinants containing yeast DNA transformed these cells and also an *E. coli leu*$^-$ strain.

Animal cell systems are also at an exciting stage of development. Some animal viruses, of which the best studied are the monkey virus SV40 and the mouse virus polyoma, can transform appropriate host cells, and recombinant DNA molecules can be made with these viral DNA molecules as vectors in just the same way as with the prokaryotic and yeast systems. Berg and his colleagues are making impressive progress in the use of SV40 and have shown that globin cDNA inserted into appropriate regions of the SV40 genome can be expressed accurately in cultures of monkey kidney cells (Mulligan *et al.* 1979). However, for correct expression, retention of part of the early region of the SV40 genome appears to be essential. Experiments with recombinants containing the genomic globin sequences are in progress at present.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Hepatitis B virus genes and their expression in *E. coli*

Expression of human virus genes in *E. coli* has been achieved recently with hepatitis B virus (Burrell *et al.* 1979), the causative agent of serum hepatitis. Fundamental studies of this virus have been seriously impaired because the virus cannot be obtained in large quantities and cannot be grown in cells in tissue culture. The virion (figure 10) is believed to be a roughly spherical particle, 42 nm in diameter, called a Dane particle, which comprises the surface, core and 'e' antigens, a viral DNA-dependent DNA polymerase, and a circular DNA molecule, r.m.m. 2×10^6; the latter contains a large gap in one strand, through which it can be labelled *in vitro* by the endogenous polymerase on incubation with radioactive deoxynucleoside triphosphates (Robinson 1977). To obtain expression of the HBV genes (Burrell *et al.* 1979), fragments of DNA isolated from Dane particles were produced by digestion with various restriction enzymes and cloned in the plasmid pBR322 at the *Pst* site by the 3′ dC, dG tailing method, as described earlier. Transformants selected on the basis of their resistance to tetracycline were shown to be sensitive to ampicillin and the presence of HBV DNA sequences was demonstrated by colony hybridization with ^{32}P-labelled DNA from Dane particles. Cells carrying the recombinant plasmids were then screened for expression of the virus core and surface antigens by the disk radioimmunoassay method (Broome & Gilbert 1978) with ^{125}I-labelled antibodies; of some 400 colonies screened, 13 gave positive reactions for the core antigen. The specificity of the core antigen activity was confirmed by several seriological reactions, and, in extracts prepared from the bacterial cells, the antigenic activity was stable for several months at −20 °C. When injected into rabbits, the bacterial extract elicited the formation of antibodies that, in the Ouchterlony (1958) diffusion assay, cross-reacted specifically with HBV core antigen from a human hepatoma (figure 11, plate 1).

Several of the cloned viral DNA segments have now been analysed in detail and much of the HBV DNA sequence determined (Pasek *et al.* 1979). The nucleotide sequence corresponding to the core antigen has been identified and, interestingly, it does not correspond with a polypeptide fused to β-lactamase as anticipated, for just within the cloned HBV sequence there is a translational stop signal separated by three nucleotides from an initiation codon. Translation of the nucleotide sequence from this point gives a polypeptide of 183 residues with a relative molecular mass of 23000, in reasonable agreement with the value of 19000 attributed to the core antigen. Nucleotide sequences that correspond with the parts of the amino acid sequence that have been determined for the surface antigen have also been located and among the clones examined at least two different sequences were found equivalent to polypeptides with two amino acid changes; this may correspond with the complex serotype of the virus preparation used for cloning (Burrell *et al.* 1979; Pasek *et al.* 1979). Expression of the surface antigen, probably as a polypeptide fused to β-lactamase has also been obtained.

The experiments with hepatitis B virus DNA not only illustrate the way in which the new genetic engineering methods can be used for detailed molecular biology studies of a viral system that is otherwise difficult to work with, but they also show the power of these methods and suggest that they may provide the preferred way for working with pathogenic organisms. Moreover, they offer the way to new and sensitive diagnostic methods and also hold promise of the development of new methods for vaccine production.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Conclusion

What, then, are the prospects for the application of genetic engineering in industrial microbiology? First, there is now available a large range of methods and systems for genetic manipulation, giving an almost limitless combination of operations for the pursuit of the isolation and expression of any particular gene. Secondly, in several cases, the yield of enzymes from bacterial cells has been increased, sometimes by very large factors indeed. Thirdly, medical applications have been clearly shown to be feasible in both diagnosis and therapy and it is perhaps in this area that the most immediate benefits of genetic engineering are to be obtained. Fourthly, there is every reason to expect very rapid progress in the accumulation of new fundamental information in cell biology, which must help applied research and development enormously.

Unhappily, the United Kingdom does not appear to be well placed to take advantage of these developments. For several years we have not been training enough people in molecular biology, microbial genetics and nucleic acid biochemistry. Investment in research is low compared with that in other countries and has been so for too many years, a point discussed in some detail by Kornberg (1978) in his Leverhulme lecture. In Universities, which is where the majority of research in this area is pursued, a succession of financial crises has led to a reduction in the amount of time and effort that academic staff can devote to research at a time when an increase in research activity should be particularly auspicious. If the new opportunities are to be exploited, more career research openings in University departments are urgently needed, as is more investment in research and development from both industrial and government sources. Activity and investment in this area are intense in the United States and other Western European countries.

References (Murray)

Arber, W. & Linn, S. 1969 *A. Rev. Biochem.* **38**, 467–500.
Beggs, J. D. 1978 *Nature, Lond.* **275**, 104–109.
Benton, W. D. & Davis, R. W. 1977 *Science, N.Y.* **196**, 180–182.
Borck, K., Beggs, J. D., Brammar, W. J., Hopkins, A. S. & Murray, N. E. 1976 *Molec. gen. Genet.* **146**, 199–207.
Breathnach, R., Mandel, J. L. & Chambon, P. 1978 *Nature, Lond.* **270**, 314–319.
Broome, S. & Gilbert, W. 1978 *Proc. natn. Acad. Sci. U.S.A.* **75**, 2746–2749.
Burrell, C. J., Mackay, P., Greenaway, P. J., Hofschneider, P. H. & Murray, K. 1979 *Nature, Lond.* **279**, 43–47.
Cameron, J. R., Panasenko, S. M., Lehman, I. R. & Davis, R. W. 1975 *Proc. natn. Acad. Sci. U.S.A.* **72**, 3416–3420.
Campbell, A. 1971 In *The bacteriophage lambda* (ed. A. D. Hershey), pp. 13–44. New York: Cold Spring Harbor Laboratory.
Chow, L. T., Gelinas, R. E., Broker, T. R. & Roberts, R. J. 1977 *Cell* **12**, 1–8.
Davies, J. & Jacob, F. 1968 *J. molec. Biol.* **36**, 413–416.
Erlich, H. A., Cohen, S. N. & McDevitt, H. O. 1978 *Cell* **13**, 681–689.
Grunstein, M. & Hogness, D. S. 1975 *Proc. natn. Acad. Sci. U.S.A.* **72**, 3961–3965.
Hayes, W. 1968 *The genetics of bacteria and their viruses* (2nd edn). Oxford: Blackwell.
Hershey, A. D. (ed.) 1971 *The bacteriophage lambda*. New York: Cold Spring Harbor Laboratory.
Hinnen, A., Hicks, J. B. & Fink, G. R. 1978 *Proc. natn. Acad. Sci. U.S.A.* **75**, 1929–1933.
Hohn, B. & Murray, K. 1977 *Proc. natn. Acad. Sci. U.S.A.* **74**, 3259–3263.
Ikeda, H. & Tomizawa, J. 1965 *J. molec. Biol.* **14**, 85–109.
Jackson, D. A., Symmons, R. H. & Berg, P. 1972 *Proc. natn. Acad. Sci. U.S.A.* **69**, 2904–2908.
Jaskunas, S. R., Lindahl, L. & Nomura, M. 1975 *Nature, Lond.* **257**, 458–462.
Jeffreys, A. J. & Flavell, R. A. 1977 *Cell* **12**, 1097–1108.
Kelley, W. S., Chalmers, K. & Murray, N. E. 1977 *Proc. natn. Acad. Sci. U.S.A.* **74**, 5632–5636.
Kornberg, H. L. 1978 The importance of being curious. *Third Leverhulme Memorial Lecture*. Liverpool: Liverpool University Press.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Mulligan, R. C., Howard, B. H. & Berg, P. 1979 *Nature, Lond.* **277**, 108–114.
Murray, K. 1976 *Endeavour* **35**, 129–133.
Murray, K. 1978 In *Genetic engineering* (ed. A. M. Chakrabarty), pp. 113–122. West Palm Beach, Florida: C.R.C. Press.
Murray, N. E. 1978 In *Genetic engineering* (ed. A. M. Chakrabarty), pp. 31–52. West Palm Beach, Florida: C.R.C. Press.
Murray, N. E. & Kelley, W. S. 1979 *Molec. gen. Genet.* **175**, 77–87.
Murray, N. E., Bruce, S. A. & Murray, K. 1979 *J. molec. Biol.* **132**, 493–505.
Ouchterlony, O. 1958 In *Progress in allergy*, vol. 5 (ed. P. Kallos & R. H. Waksman), pp. 1–78. Basle and New York: Karger.
Pasek, M., Gilbert, W., Zink, B., Schaller, H., Mackay, P., Leadbetter, G. & Murray, K. 1979 *Nature, Lond.* **282**, 575–578.
Roberts, R. J. 1976 *C.R.C. crit. Rev. Biochem.* **4**, 122–164.
Roberts, R. J. 1980 *Nucleic Acids Res.* **8**, r63–r80.
Robinson, W. S. 1977 *A. Rev. Microbiol.* **31**, 357–377.
Sanzey, B., Mercereau, O., Ternynzk, T. & Kourilsky, P. 1976 *Proc. natn. Acad. Sci. U.S.A.* **73**, 3394–3398.
Seeburg, P. H., Shine, J., Martial, J. D., Ivarie, R. D., Morris, J. A., Ulrich, A., Baxter, J. D. & Goodman, H. M. 1978 *Nature, Lond.* **276**, 795–798.
Sgaramella, V., van de Sande, J. H. & Khorana, H. G. 1970 *Proc. natn. Acad. Sci. U.S.A.* **67**, 1468–1475.
Sharp, P. A., Sudgen, W. & Sambrook, J. 1973 *Biochemistry, N.Y.* **12**, 3055–3063.
Skalka, A. & Shapiro, L. 1977 *Gene* **1**, 65–79.
Smith, H. O. & Nathans, D. 1973 *J. molec. Biol.* **82**, 419–423.
Sternberg, N., Tiemeier, D. C. & Enquist, L. 1977 *Gene* **1**, 255–280.
Sugino, A., Goodman, H. M., Heyneker, H. L., Shine, J., Boyer, H. W. & Cozzarelli, N. R. 1977 *J. biol. Chem.* **252**, 3987–3994.
Sutcliffe, J. G. & Asubel, F. M. 1978 In *Genetic engineering* (ed. A. M. Chakrabarty), pp. 83–111. West Palm Beach, Florida: C.R.C. Press.
Tilghman, S. M., Tiemeier, D. C., Seidman, J. G., Peterlin, B. M., Sullivan, M., Maizel, J. V. & Leder, P. 1978 *Proc. natn. Acad. Sci. U.S.A.* **75**, 725–729.
Villa-Komaroff, L., Efstratiadis, A., Broome, S., Lomedico, P., Tizard, R., Naber, S. P., Chick, W. L. & Gilbert, W. 1978 *Proc. natn. Acad. Sci. U.S.A.* **75**, 3727–3731.
de Waard, A., Korsuize, J., van Beveren, C. P. & Maat, J. 1978 *FEBS Lett.* **96**, 106–110.
de Waard, A., van Beveren, C. P., Duyresteyn, M. & van Ormondt, H. 1979 *FEBS Lett.* **101**, 71–76.
Wilson, G. G. & Murray, N. E. 1979 *J. molec. Biol.* **132**, 471–491.

Discussion

B. S. HARTLEY, F.R.S. (*Department of Biochemistry, Imperial College, London SW*7). When did this work on cloning the hepatitis viral DNA begin?

K. MURRAY. Thirteen months ago, and we have had an excellent spirit of cooperation in collaberative work with Dr Marmion, Dr Gilbert and Dr Schaller. The work was supported by commercial venture capital from Biogen.

E. G. BEVERIDGE (*School of Pharmacy, Sunderland Polytechnic, Sunderland SR*1 3*SD, U.K.*). To what extent are the U.K. G.M.A.G. regulations helpful or restrictive to this type of research?

K. MURRAY. At present the regulations are restrictive, but the situation is improving. This work on hepatitis virus presents an interesting case in point; the work that I have described was carried out at M.R.E. (Porton) under 'category four containment', which, as some of you will know, is very troublesome. At that stage this work would not have been permitted in the U.S.A.; however, with the revision of both the U.S. and the U.K. guidelines, this experiment now comes under 'category one containment' in the U.S. and 'category two' in the U.K. Thus the relative ease with which such work can be done in the two countries has been reversed. If the work had been permitted in the U.S. from the outset, the work would already have been carried out there, or at least the magnitude of the competition would have been too great.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

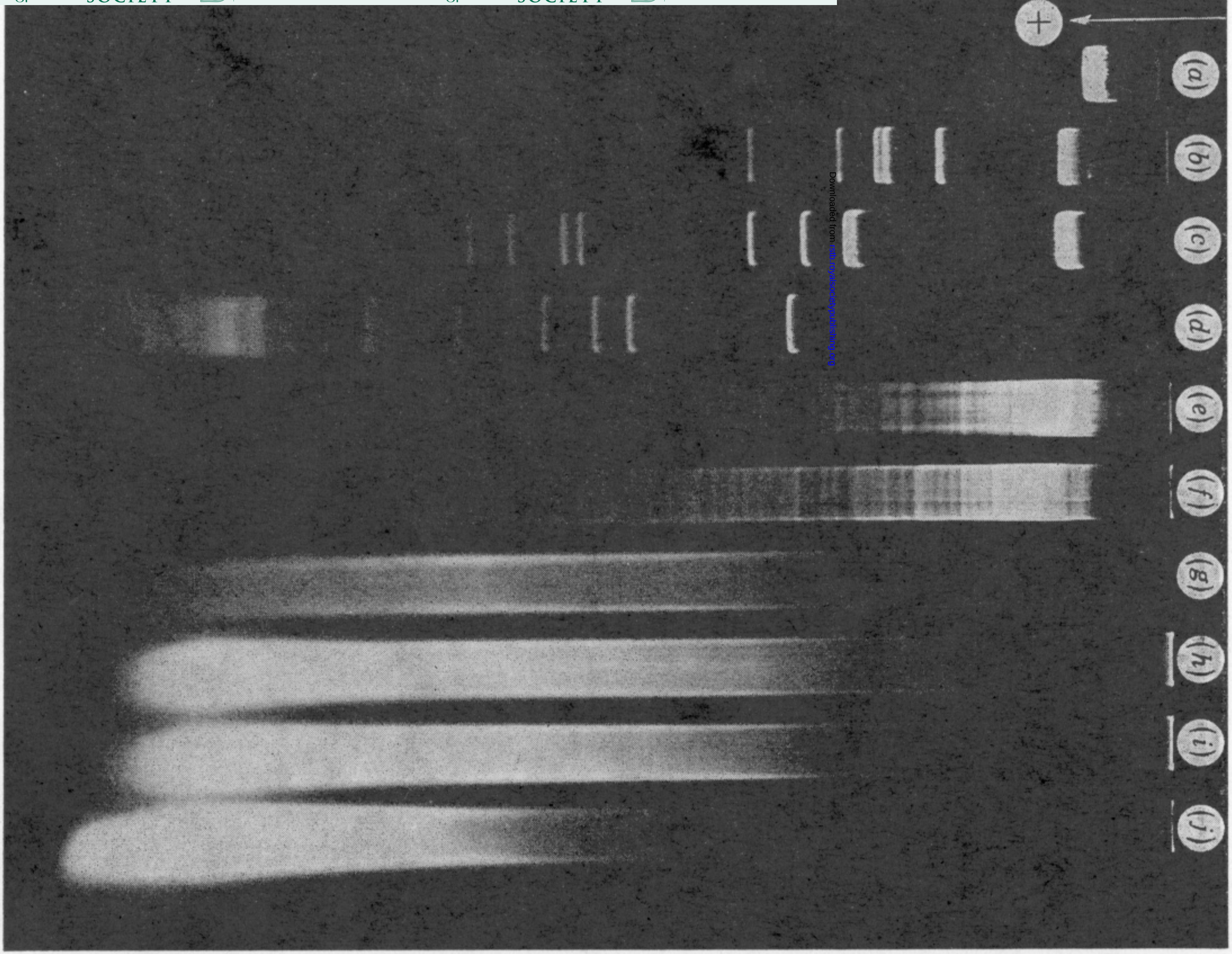

FIGURE 3. For description see opposite.

FIGURE 4. For description see opposite.

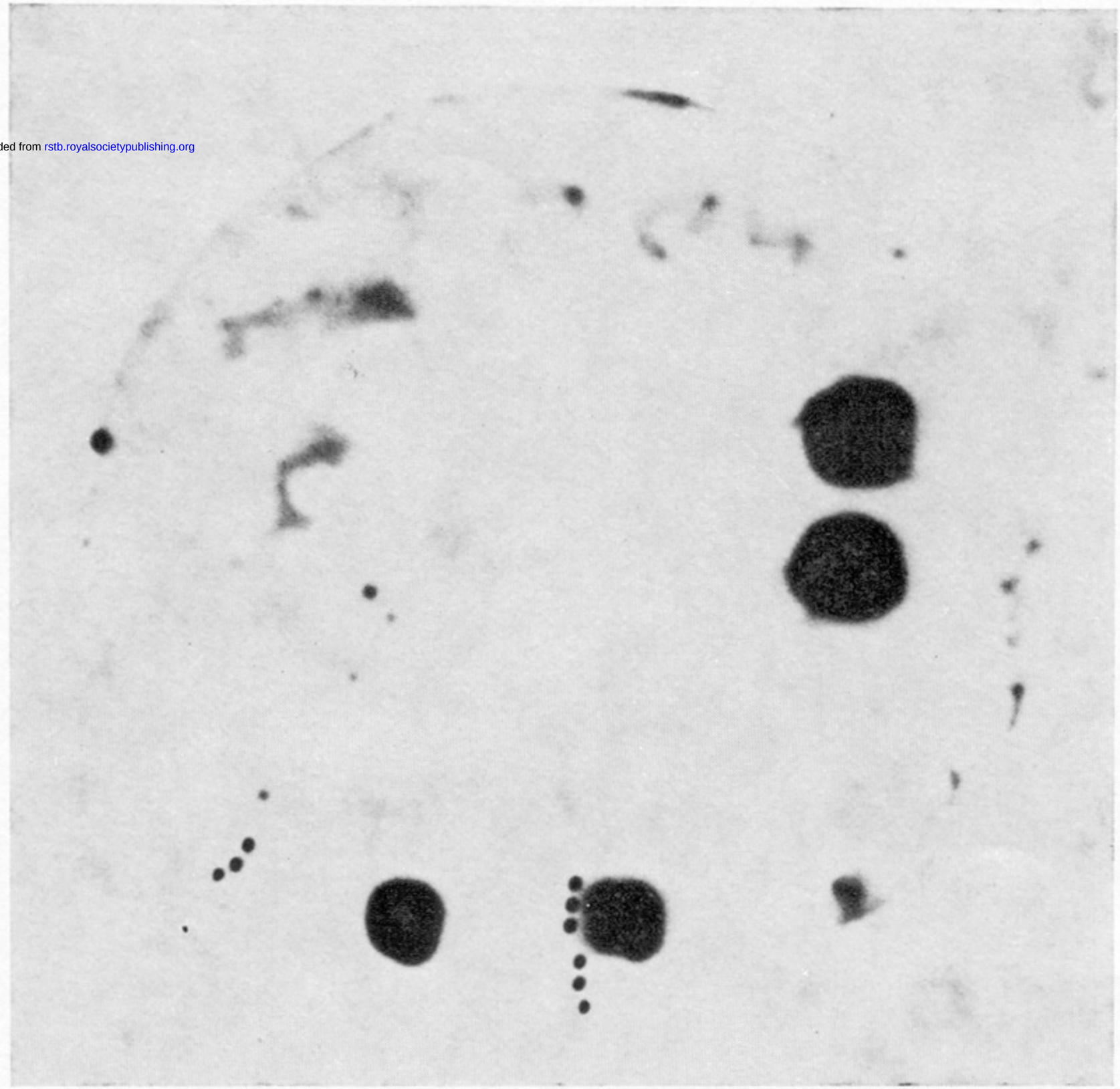

FIGURE 9. The radioimmunoassay for detection of bacterial colonies producing an antigenic polypeptide. The example (from Burrell *et al.* (1979)) shows that 4 out of the 52 colonies imprinted upon the plastic disk coated with anti-HBc gave positive reactions when the disk was subsequently treated with ^{125}I-labelled HBc antibodies.

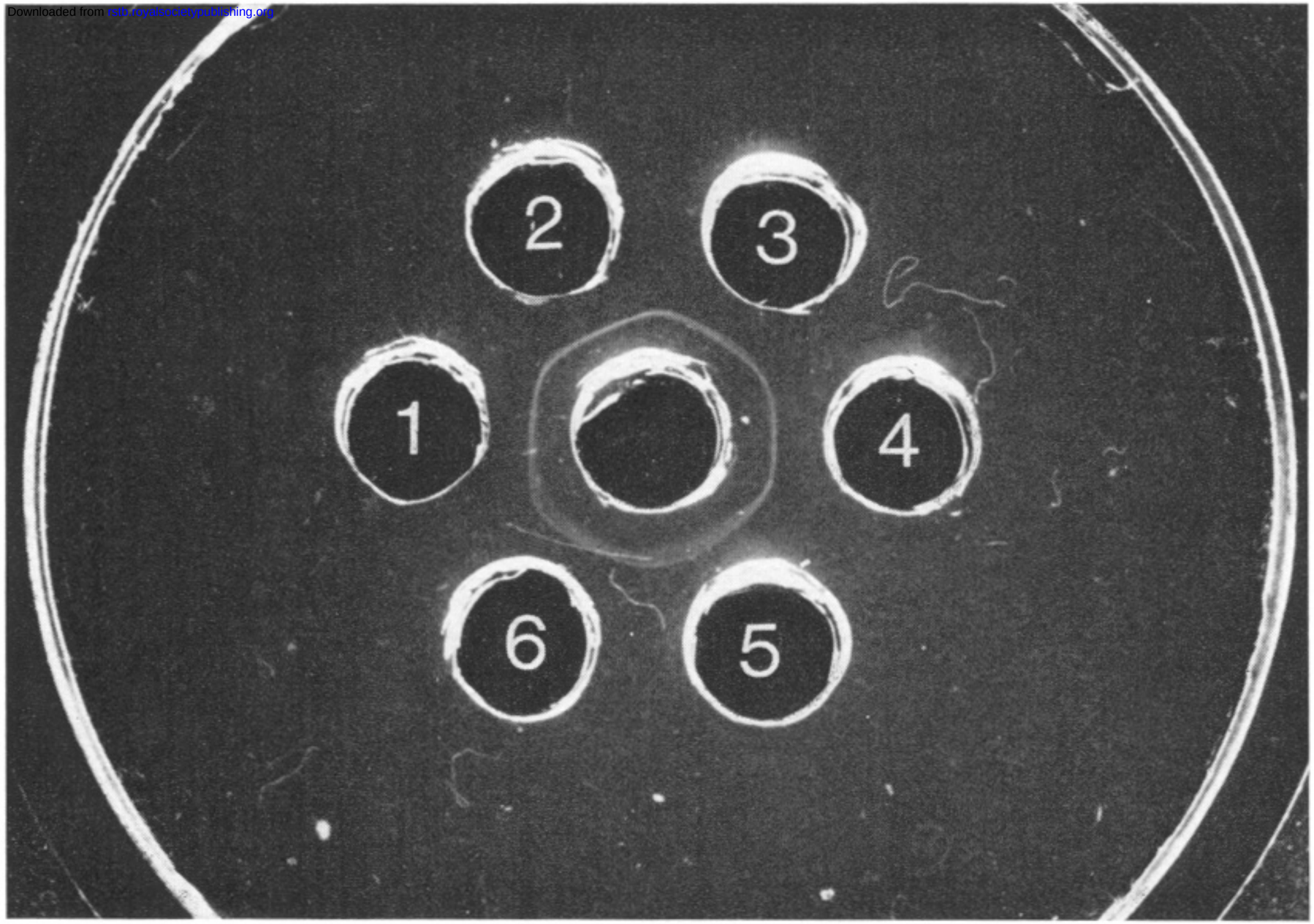

FIGURE 11. Comparison, by the immunodiffusion method, of human antibodies to HBcAg with those from rabbits injected with HBcAg made in *E. coli*. The centre well of the gel (9 g/l agarose in 0.01 M Tris–HCl pH 7.2, 0.001 M EDTA, 0.1 M-NaCl) contained partially purified HBcAg derived from human liver. Wells 1 and 4 contained human anti-HBc at a dilution of 1 in 30; wells 2 and 3 contained undiluted serum from rabbits injected with the unfractionated extract of bacteria carrying clone pHBV139; wells 5 and 6 contained undiluted rabbit serum raised against HBcAg from the same bacterial extract, but fractionated on a column of Sephadex G50 (Pasek *et al.* 1979).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES