GENES, MICROBES AND MAN

BY

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Mr. Vice-Chancellor, Members of the University, Ladies and Gentlemen, a one year course in Microbiology was initiated, at the second year level, at Rhodes in 1960 as a result of the foresight of Professor E. S. Twyman. Only in 1968 was his ambition to have degree and honours courses in Microbiology realised. It was because I would be working with Professor Twyman that I decided to return to Rhodes in 1968, and I wish to acknowledge and thank him for all the assistance and expert guidance he has given me, first as a student and more recently in establishing Microbiology. I was a member of that first Microbiology class in 1960 and I hope it gives Professor Twyman some pleasure to have one of those pioneering students, although by no means the brightest, standing here this evening.

Although Microbiology at Rhodes is only 5 years old, we have achieved some considerable success in our research projects which have resulted in both international and local recognition. This success has been due to the co-operation and calibre of the Microbiology staff, DONALD HENDRY and SHEILA MELDRUM, and the post-graduate students in Microbiology. Some of the work I shall be referring to this evening has been done in conjunction with them.

One of my lesser known activities on this campus has been as Warden of "Botha House." This sometimes onerous task has been handled entirely by my wife allowing me to spend long hours in the laboratory and equally long hours trundling around a squash court. She can best be described as the first Lady Warden of a men's residence. The moments I have enjoyed most are when parents arrive on our doorstep and they ask if they could see her Daddy, the Warden Professor!

My training as a Microbiologist and, more particularly as a Microbial Geneticist, started in earnest at Oxford University and I was fortunate to have as my supervisor Dr. E. A. Bevan, now Professor of Plant Biology and Microbiology at Queen Mary College, London University. He is a most stimulating and inspiring university teacher and was responsible

for introducing me to the fascinating field of Molecular Genetics, aspects of which I shall discuss this evening.

The study of Microbiology received a tremendous boost with the discovery of sex in bacteria by Lederberg and Tatum in 1946. With the discovery of genetic recombination in both bacteria and bacteriophages (Visconti & Delbruck, 1953), the potential of microbes as tools in molecular genetics was recognised and rapidly exploited.

Two sexual types of bacteria are known and are illustrated in Fig. 1. The "male" cell is characterised by the presence of sex pili and a sex factor (F) in the cytoplasm which controls the "male" or donor condition. The bacterial chromosome and the sex factor, which is approximately 1/200th the size of the chromosome, are structurally similar in that each element is a single circular double stranded DNA molecule. F possesses its own replicon, which is a set of genes controlling replication and is able to replicate autonomously in the cytoplasm. It is possible for a cell to have more than one sex factor and the average number is between 2-5 per cell. The sex pili are distinct from the flagella and other pili which have been left out of the diagrams for clarity. "Male" bacteria can be distinguished experimentally from "female" bacteria by a special class of male specific bacteriophages (bacterial viruses) which can only attach to sex pili and thus only destroy "male" bacteria.

The sex pili are implicated in the formation of mating pairs between F^+ and F^- bacteria and the formation of a conjugation tube (Fig. 2). The sex factor in the F^+ donor bacterium replicates prior to one of the daughter DNA strands migrating via the tube to the recipient (F^- female) which then becomes a "male" bacterium. As a result of conjugation and the transfer of the sex factor, a mating between an F^+ and an F^- bacterium gives rise to two "male" F^+ cells. Since the sex factor replicates autonomously and is transferred faster than the cells divide, all the F^- recipient cells in a mixed culture will ultimately be converted to F^+ donors. This epidemic spread of F^+ through a bacterial population is a most important aspect of conjugation, as it concerns the spread of antibiotic resistance in bacteria. The genes carried by the F factor which control the ability to mate are referred to collectively as a conjugon.

A sex factor can also recombine with other sex factors or cytoplasmic DNA as well as with the chromosome. The set of genes controlling the ability to carry out recombination is known as a recon.

FIG 1

SEXUAL TYPES OF E.coli

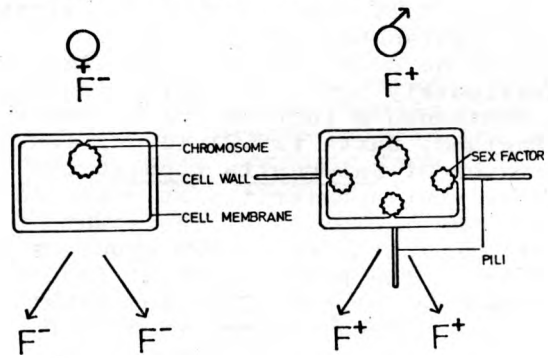


FIG 2.

CONJUGATION

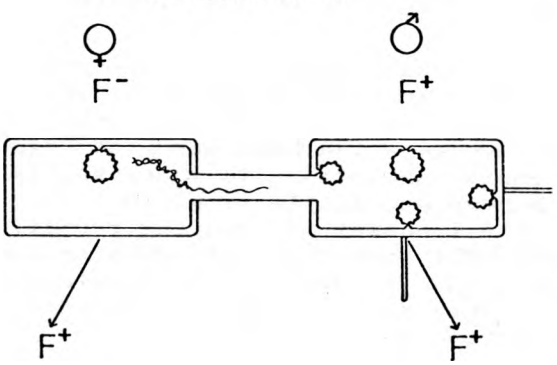
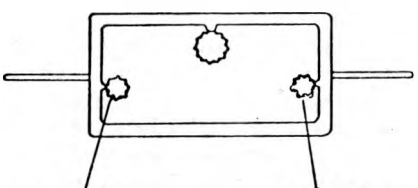


FIG. 3.

R FACTOR



F FACTOR
(i) REPLICON
(ii) RECON
(ii) CONJUGON

R FACTOR
(i) REPLICON
(ii) RECON
(iii) CONJUGON
(iv) UP TO 7 GENES FOR DRUG (ANTIBIOTIC) RESISTANCE.

Drug (antibiotic) resistance factors or R factors (Fig. 3) are similar to F factors in that they are cytoplasmic 2-DNA molecules consisting of a replicon, recon, conjugon and up to 7 structural genes for drug resistance. These might include resistance to streptomycin, chloramphenicol, tetracyclines, sulphonamides, kanamycin, neomycin, penicillins and trimethoprim. They are characterised by epidemic spread and the alarming rate of evolution and spread of R factors can be illustrated by three reported examples.

The first R factor containing bacterium, a Shigella strain, was isolated in Japan in 1957 (Watanabe, 1966). Prior to this date, there were virtually no drug-resistant strains of Shigella but, in 1964, approximately 50% of Shigella isolates in 3 major Japanese cities contained R factors for at least 4 commonly used drugs. In England, Datta (1962) identified the first R factor among strains of Salmonella typhimurium isolated during an outbreak of gastro-enteritis at a London hospital. By January, 1965, the incidence of resistant strains of this species at the hospital rose from about 3% to 61% (Anderson and Lewis, 1965). The pharmaceutical industry released the new drug Trimethoprim onto the English market in 1969. The advertisements for the drug claimed that no R factor bacterial strains would ever be isolated since the drug contained two active ingredients which affected, each in a different manner, the same metabolic step. It was considered that the chance of obtaining resistance was minimal since resistance would involve simultaneous resistance to both active ingredients. The first R factor strains conferring transferable resistance to Trimethoprim were isolated in 1971 - only 2 years after release of the drug (Fleming, Datta and Grüneberg, 1972).

In Microbiology at Rhodes, we have undertaken research on R factors. Rhodes is fortunate in being situated close to the Ciskei and Transkei because these areas contain urban Xhosa populations near East London which have been much exposed to drugs, as well as remote populations in inaccessible parts of the Transkei which have had little or no contact with drugs. Furthermore, the Transkei is changing rapidly and these remote populations will soon come into greater contact with drugs. It is important to monitor any developments regarding drug resistance as this may provide further information as to its evolution and control.

A survey of the incidence of drug resistant and R factor strains of coliform bacteria from stool samples from urban and remote Xhosa populations indicated the strikingly high degree of resistance (82,9%) and R factors (61,5%) in strains from urban Xhosa (Woods, Marcos and Hendry, 1972). This could be the result of the use of drugs among an overcrowded, poor and uneducated population with inadequate domestic hygiene facilities and sewage treatment. The unhygienic neighbourhood would serve as a reservoir for resistant strains.

Due to the low level of education and unfavourable economic position, the situation would be aggravated by patients failing to complete a course of drugs on discharge from hospital or on feeling better. These results illustrate the selective force of antibiotics and the undesirable effects that can arise from the uncontrolled use of drugs in crowded, underdeveloped countries.

In order to test the hypothesis that the unhygienic neighbourhood in the urban township was serving as a reservoir for resistant strains, a second R factor study was undertaken, involving a comparison between an urban Xhosa population and a remote Xhosa population, and their respective environments (soil, water, drains, sewage, cattle kraals) (Marcos and Woods, 1973). The results shown in Table 1 indicate the striking difference between the incidence of resistance and R factors among bacteria from urban and from remote communities and their environments. It is apparent that R factors exist and survive in bacteria from the environment and that the crowded township studied is serving as a reservoir for R factors. Careful monitoring of transferable drug resistance in overcrowded, unhygienic and underdeveloped communities is essential if the full benefit of modern antimicrobial therapy is to be derived.

TABLE 1. INCIDENCE OF RESISTANCE AND R FACTORS IN COLIFORM BACTERIA FROM URBAN AND REMOTE COMMUNITIES

	No. of strains tested	R*	TR†
Remote population	90	18,9	11,8
Remote environment	51	25,5	0
Urban population	67	68,6	47,8
Urban environment	83	66,3	23,1

*R = % resistant strains

†TR = % resistant strains which carry R factors

The Xhosa township where this work was carried out is situated on the slopes of a ridge near East London at the bottom of which is the beautiful Buffalo Dam, East London's main water supply. Storm water drainage is often not adequate and, after heavy rainfall, much of the storm water finds its way into the dam, taking with it resistant, harmful bacteria from the unhygienic township environment. This pollution of East London's main water supply by R factor strains is very serious since it has been recently shown by the National Institute for Water Research at the C.S.I.R. that

harmful bacteria carrying R factors are resistant to normal water purifying treatments (i.e. chlorination).

It is interesting to note that in Britain the main reservoir of Salmonella typhimurium strains carrying R factors was traced to intensive cattle breeding farms where the animals were crowded and hygienic conditions were unsatisfactory (Hayes, 1968). The farmers controlled infectious bacterial diseases by routinely adding antibiotics to the feed. This worked well for a while but at the same time provided an ideal environment for the selection and rapid evolution of R factor strains. These strains could cause serious epidemics among both cattle and man as they could not be controlled by antibiotics to which they were now resistant. The deteriorating situation resulted in a British government enquiry under the chairmanship of Swann in 1969. The Swann report recommended that routine use of antibiotics in feed be discontinued.

I have deliberately pointed out the dangers of selection and evolution of resistant strains by the indiscriminate use of antibiotics. However, I am not advocating the abolition of the use of antibiotics. We all owe a great debt to the development of antibiotics for therapeutic purposes. Many of the fatal bacterial diseases which were prevalent at the beginning of this century have been controlled by antibiotics to the point of extinction. In order to avoid the problems of resistance and to preserve the benefits of antibiotic therapy for mankind, I suggest the strict control and phased release of antibiotics. In the same way that the incidence of antibiotic resistant strains reflects antibiotic use, the corollary also applies in that withdrawal leads to a decrease in the number of resistant organisms encountered (Richmond, 1972). It is noteworthy that, during the rapid evolution of R factor strains in Japan (see above) antibiotics were not controlled at all and were available without prescription. The political and economic problems of implementing such a drug rotation scheme are obvious but must be overcome. The statistics regarding the use of antibiotics in Britain for the year 1967 are staggering and indicate the size and economic importance of the Pharmaceutical industry, which may be difficult for any government to control. The information published in the Report of the Swann Committee (1969) shows that in the year 1967, $\pm$ 240 000 Kg of antibiotics were used in Britain for human medicine and another 168 000 Kg for veterinary purposes.

R factor transfer between bacteria isolated from faecal specimens occurs relatively easily in the laboratory under aerobic conditions. It is not clear how this transfer occurs in the anaerobic environment of the lower gastro-intestinal tract, as in vitro studies by Mitsuhashi (1965), with an R factor from a Shigella flexneri strain, indicated that conjugation was completely inhibited by anaerobic conditions. However, in vivo transfer has been demonstrated

with R factors (Guinée, 1970). Because of this contradictory situation, we investigated R factor transfer by facultative Escherichia coli strains under aerobic and anaerobic conditions (Moodie and Woods, 1973). We have demonstrated that R factor transfer is possible in the anaerobic environment of the lower gastro-intestinal tract. Our result differs from that of Mitsuhashi (1965) and provides support for previous reports of in vivo R factor transfer and in vitro F factor transfer under anaerobic conditions (Stallions and Curtiss, 1972).

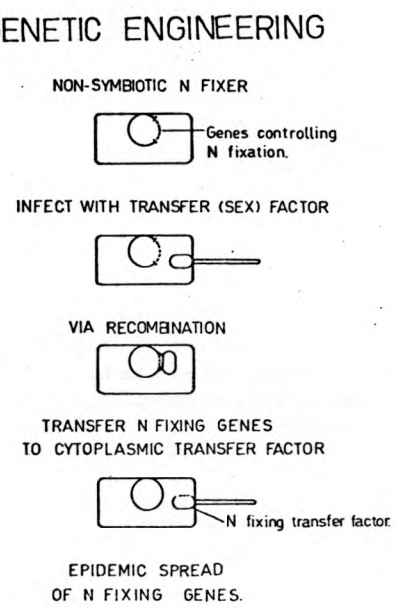
Although the majority of bacteria inhabiting the human gut are obligate anaerobes, the study of these bacteria is only at the pioneering and developmental stage. Anaerobes are also being increasingly implicated in disease and we have initiated research projects to investigate this interesting and important group of bacteria. As the non-pathogenic anaerobes form the majority of the bacteria of the gut, they may act as a reservoir of R factors and play a most important rôle in the spread of drug resistance. As yet R factor transfer has not been demonstrated in anaerobes and we are investigating the possibility of gram negative anaerobes harbouring R factors.

Microbiologists have recognised the potential of conjugation and the horizontal or epidemic spread (as opposed to vertical or parent offspring spread) of genes as a tool for genetic engineering. Those of you who are farmers or keen gardeners, will know of the importance of nitrogen or nitrogenous fertilisers. The chronic world-wide shortage of dietary protein is fundamentally a problem of inadequate nitrogen fixation and reduction to ammonium, the raw material required for building all nitrogenous compounds (e.g. proteins) of the cell.

Certain plants, the legumes, are able to fix nitrogen directly from the air where it abounds. This important property of legumes is due to a symbiotic relationship with nitrogen fixing bacteria (Rhizobium species) which are located in root nodules. Farmers utilise legumes in rotation with cereal crops in order to fertilise the soil. A limited number of free-living soil bacterial species are also able to fix atmospheric nitrogen but unfortunately, they are only a small minority of the total soil bacterial population. The ability to fix nitrogen depends on a complex enzyme system called nitrogenase, which binds nitrogen gas and reduces it to ammonium (Streicher, Gurney and Valentine, 1972).

By genetic engineering, microbiologists envisage the infection of free-living nitrogen fixers with transfer factors or sex factors (Fig. 4). Following recombination between the nitrogenase genes and the transfer factor, the nitrogenase genes would be integrated with the transfer factor and

FIG 4.



located in the cytoplasm. In this state, the nitrogenase genes would be transferred along with the transfer factor and show the characteristics of epidemic spread. The transformation of the majority of soil bacteria to be able to fix nitrogen could then be accomplished.

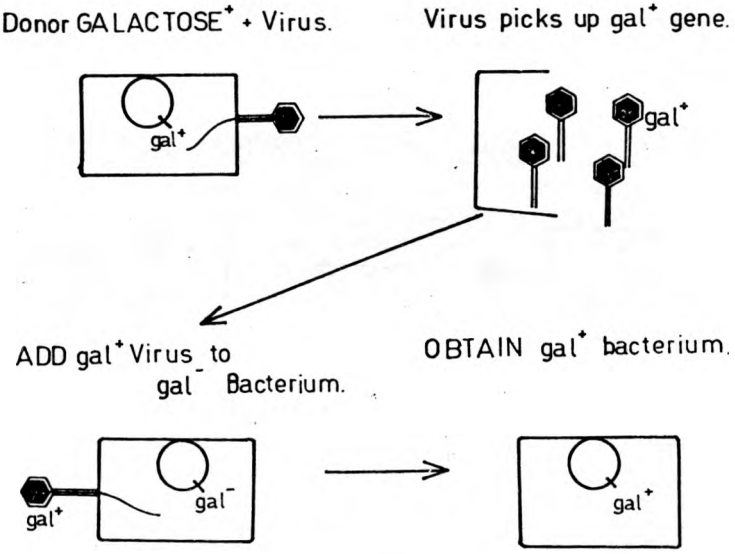
Although it would be wrong and misleading to suggest that the world fertiliser problems are over, a start on this type of genetic engineering has already been made by Postgate and his colleagues at the ARC Unit of Nitrogen Fixation at the University of Sussex (Dixon and Postgate, 1972). They have successfully transferred the nitrogen fixation genes (nif) from a strain of Klebsiella pneumoniae, a bacterium which under anaerobic conditions can fix nitrogen, to both a second strain of this species which lacks this ability and to a strain of the common gut bacterium Escherichia coli. The eventual aim of this type of genetic engineering is to transfer the nif genes to plants which would then have the inherited capacity to fix atmospheric nitrogen in sufficient amounts to meet their own needs.

The transfer of bacterial genes to higher organisms is not as far fetched a proposal as might appear at first sight. DNA is the universal genetic material and such a transfer has been successfully accomplished between bacterial genes and animal cells.

FIG. 5.

TRANSDUCTION.

BACTERIUM to BACTERIUM



There is a second genetic system in bacteria known as transduction (Fig. 5). Transfer of genetic material from a donor to a recipient cell is accomplished, without cell contact, by means of a bacterial virus acting as the carrier of the DNA. It is possible to grow bacteriophages on a bacterial strain which has the genes enabling it to utilise the sugar galactose (gal^+). The bacteriophages are then isolated and a certain proportion of them will have picked up the gal^+ genes. After infection of a gal^- bacterial strain which can not utilise galactose, a small proportion of the gal^- bacteria will receive the phages carrying gal^+ and be transformed (transduced) to gal^+

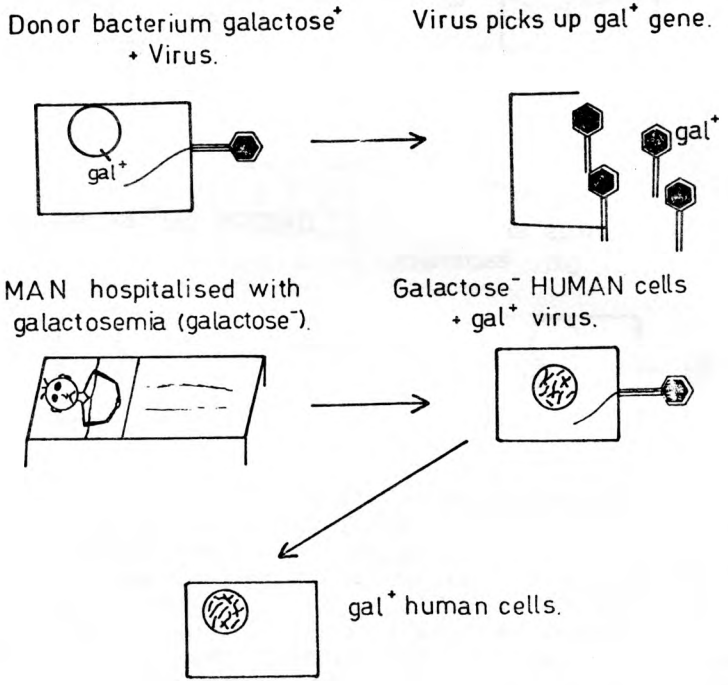
Man has the identical galactose genes as the gal^+ bacterium. People lacking the gal^+ genes develop the disease galactosemia and may be hospitalised. Genetic surgery envisages the curing of gene controlled diseases by replacing the defective genes with new DNA. Since bacteria have the same genes for galactose utilisation as human cells, they are an obvious and convenient source of new and functional gal genes for galactosemia sufferers. The difficulty is isolating these specific genes and getting them integrated into the human cell. However, these problems have been overcome and, in 1971, Merril, Geier and Petricciani amazed the scientific world by successfully transforming (transducing) human fibro-

blast cells cultured from a galactosemia patient with a bacteriophage carrying bacterial gal$^+$ genes (Fig. 6). The bacterial gal$^+$ genes were stably inherited and functioned normally in the human cells.

FIG. 6.

TRANSDUCTION.

BACTERIUM to MAN.



Although this transformation of human cells by bacterial genes has only been done with human cells growing in tissue culture and not as yet with the living patient, it heralds the dawn of genetic surgery. This is further emphasised by the first synthesis de novo of a gene in 1970 by Khorana and his large research team. Although they have synthesised one of the smallest genes (the gene for yeast alanine transfer RNA consisting of 77 base pairs as compared with 900 for an average gene producing a protein, Khorana has established techniques that will, doubtless, spread and become automated with the same remarkable speed as those for sequencing both proteins and nucleic acids.

These steps are being taken towards genetic surgery of humans but the obstacles are many and great. However, I

quote Marshall W. Nirenburg, Nobel prize winner for solving the genetic code

> "I have little doubt that the obstacles will eventually be overcome. The only question is, when? My guess is that cells will be programmed with synthetic messages within 25 years. If efforts along these lines were intensified, bacteria may be programmed within 5 years.
>
> The point which deserves special emphasis is that man may be able to programme his own cells with synthesized information long before he will be able to assess adequately the long-term consequences of such alterations, long before he will be able to formulate goals and long before he can resolve the ethical and moral problems which will be raised".

(in "The Biological Time Bomb" by Taylor, 1968, p.183).

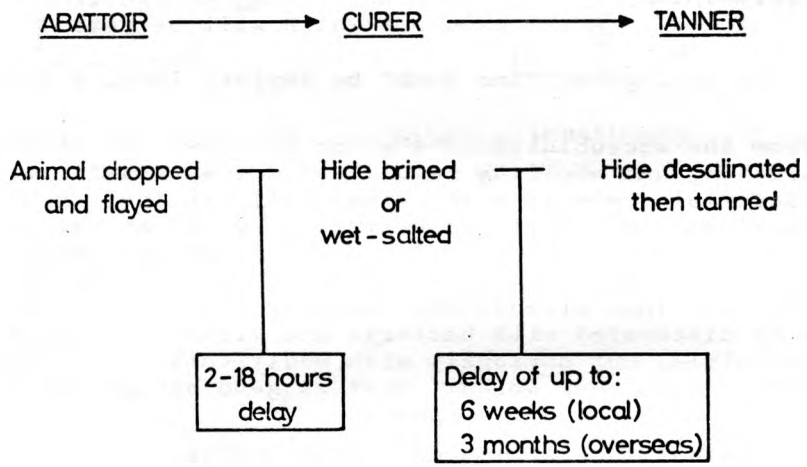
From the microbiologist's point of view, one of the most interesting and exciting results of the successful transfer of bacterial genes into an animal cell, is that they are transcribed and translated correctly by the animal systems. The animal systems therefore recognise the same signals (e.g. correct starting and stopping positions) as the bacterial cell. This strengthens the microbiologist's belief that what is discovered with bacteria and viruses may also apply in principle, but obviously with modifications, in higher organisms. In other words: what is good enough for bacteria is good enough for the elephant.

At present, one of the most challenging fields of research in both microbes and higher organisms is that of gene regulation and differentiation. The regulation of some 6 bacterial operons (lactose, galactose, arabinose, tryptophan, arginine and histidine) is beginning to be understood. These operons all have aspects in common regarding their control mechanisms but they each have unique features. We have embarked on a research programme concerning the regulation of the collagenase gene in bacteria. This gene is also present in human cells. The enzyme collagenase is the only enzyme which can degrade collagen and this molecular genetics project aimed at elucidating the control of the collagenase gene arose out of an important practical and industrial research project.

In conjunction with Dr. D. R. Cooper and the Leather Industries Research Institute, we are investigating the cause of leather decay in hides which is attributed to bacterial degradation of collagen. We have shown that hides are contaminated with halotolerant bacteria which are able to destroy collagen under aerobic conditions (Woods, Welton, Thomson and Cooper, 1972 a & b). The discovery of aerobic collagenolytic

bacteria is particularly significant because bacterial collagenolysis (with one exception) was previously only known to occur under anaerobic conditions (Thomson, Woods and Welton, 1972; Welton and Woods, 1973). Prior to this work, halotolerant bacteria were not known to be collagenolytic. We have been able to pinpoint the stage when the hides are most susceptible to bacterial decay (Woods, Rawlings, Cooper and Galloway, 1973). This stage is immediately after flaying and before curing (Fig. 7). Although the time might seem relatively short (2-18 hrs), it is quite sufficient for considerable bacterial growth and collagenase production. The hide and skin, and leather industries have benefitted from this work and are taking steps to ensure the prevention of bacterial damage to raw hides.

FIG. 7



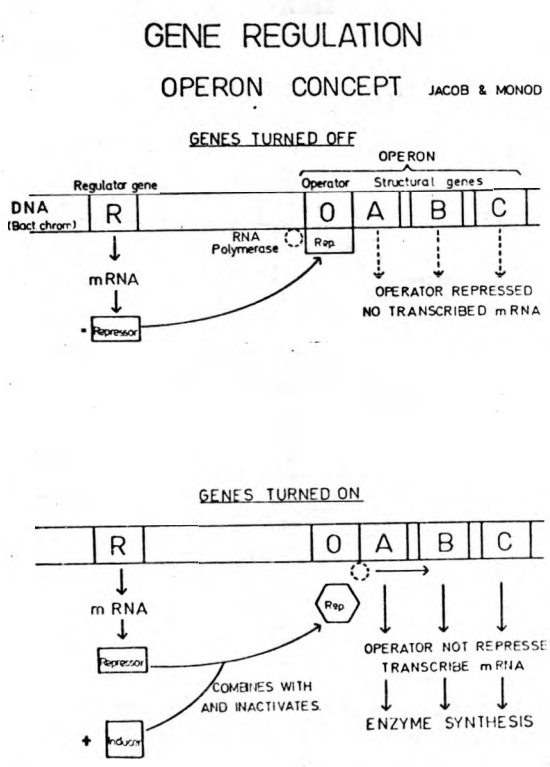
We are at present purifying and studying the bacterial collagenase enzymes with 3 main objects in mind. Firstly, the development of specific inhibitors of cell-free collagenase with a view to the elimination of curing with salt. The disposal of used salt from curers is a major pollution hazard and the elimination of salt for curing hides is of immediate importance.

Secondly, collagenases are becoming increasingly important in medicine and tissue culture techniques (Mandl, 1972). At present, the best source of commercial collagenase is *Clostridium histolyticum*, several strains of which produce collagenase anaerobically. Clostridial collagenase has disadvantages in that anaerobic conditions are required for the production of the enzyme and as clostridial toxin is so lethal, it is necessary to purify the enzyme carefully before it can be used on humans (Sizer, 1972). We are investigating the optimum conditions for the production of collagenase by our

non-pathogenic bacteria with a view to utilising them as a source of medicinal collagenase.

Thirdly, we are interested in the control of the collagenase operon both from the academic and practical aspects. Development of a specific inhibitor of the induction of the collagenase operon would result in a two-edged sword for the fight to prevent hide and leather decay. Inhibition of the production of the enzyme as well as inhibition of the enzyme itself would then be possible.

FIG 8.

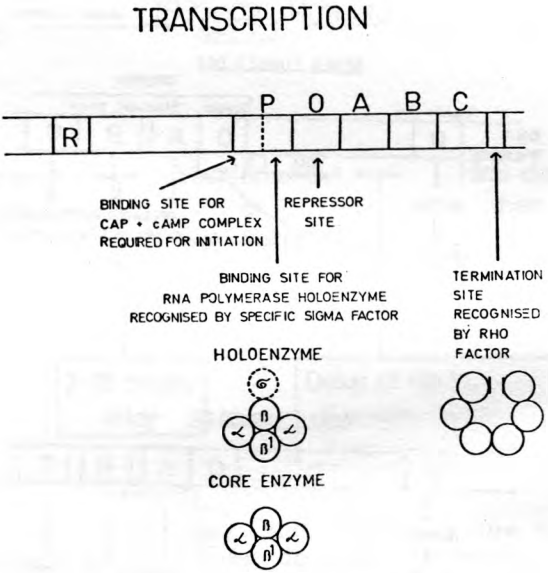


Jacob and Monod (1961) proposed the operon concept (Fig. 8) as a model for gene regulation. It would be fascinating to determine whether collagenase is controlled by a similar mechanism. As yet, nothing is known about the regulation of collagenase. It is not known how many genes are involved in the degradation and uptake of collagen by bacteria

or whether these genes are arranged in one or more operons. The nature of the substance which induces the bacterium to produce collagenase is interesting as it can not be the huge collagen molecule itself. Perhaps another protease enzyme degrades a telopeptide which then acts as the inducer.

The operon concept as outlined in Fig. 8 has been proved experimentally for the lactose operon. A cytoplasmic repressor protein has been isolated and it has been shown to bind specifically with an inducer and the operator gene. The latest developments regarding transcription are summarised in Fig. 9.

FIG 9



During this lecture, I hope that I have given you some idea of how much we know about genes and the exciting experiments being attempted by microbiologists. I wish again to stress the importance of cytoplasmic genes and viruses and their ability to promote "horizontal gene transfer" as opposed to the conventional vertical or parent to offspring genetics. Both conjugation and transduction are mechanisms whereby the characteristics of a bacterial population can be altered much more rapidly than by normal mutation and Mendelian inheritance.

The importance and nature of horizontal gene spread as opposed to Mendelian inheritance is only beginning to be realised. At a symposium on the biology and control of

ticks in southern Africa in 1969, I described the spread of genes in bacteria by conjugation and transduction and suggested the idea that the apparent rapid evolution in resistance to insecticides in ticks may be spread by a similar mechanism mediated by a virus. Although most participants thought my suggestion absurd, I was most encouraged by an article in "Nature" 1970, and I quote:

> "Extrachromosomal hereditary elements may be very much more important than present genetic convention supposes and Grüneberg's hypothesis that extranuclear entities are responsible for differentiation in skeletal patterns between sublines of mice is intriguing."

(Beardmore, 1970).

Although other alternatives cannot be excluded, I wish to suggest that it is more than intriguing and deserves investigation.

Thank you, Mr. Chairman.

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