BIOCHEMISTRY, GENETICS AND SOCIETY

Prof. D. Robinson

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REDE

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door

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Mijnheer de Rector Magnificus,

Mijne Heren van het College van Bestuur,

Mijnheer de Secretaris van de Universiteit,

Dames en Heren Hoogleraren en Leden van de Wetenschappelijke Staf,

Dames en Heren Studenten,

en voorts U allen, die door Uw aanwezigheid blijk geeft van Uw belangstelling,

Biochemistry, Genetics and Society

The audience may well ask - as I have asked myself - "What is the relevance of the appointment of an academic Biochemist to the Tinbergen chair?" I do, however, see a certain irony that at this time the economic state of British Universities is going into a rapid decline, so that one wonders if the third world - academically speaking - does not begin as soon as you head west from the Hook of Holland.

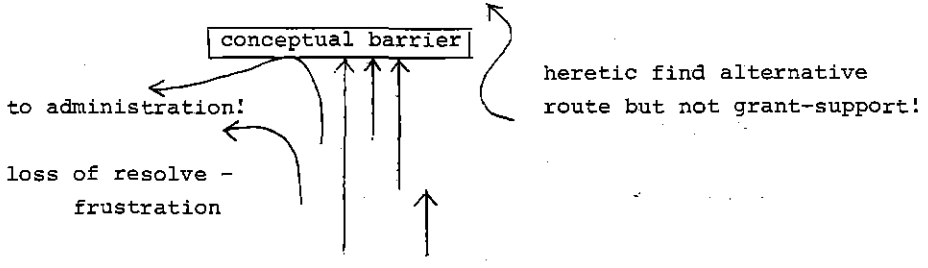
There is a story circulating in the U.K. of the professor who had need to call in a plumber to repair his water system. The job was done to his satisfaction but he was aghast when he received the bill. "Good gracious" he said "I don't get paid so much for being a professor", to which the plumber is reported to have replied "Nor did I when I was a professor".

The moral seems to be that we need to try to evaluate our worth to the community, and to justify our existence - not to say our academic freedom, and economic recessions, like executions serve to concentrate the mind wonderfully. The problem is to decide exactly who is competent to make the value judgement on an academic research programme. Certainly not the scientist alone since one of the features of enquiry after basic knowledge is that the new questions that will arise, and the eventual outcome cannot be predicted in other than very broad and imprecise terms: "A man never mounts higher than when he knows not whither he is going" (Cromwell).

The consequences of any discovery are often not evident until long after the event in many cases and therefore it is short-sighted - even if understandable, for politicians to seek immediate material results and to attempt to optimise research effort to this end. No amount of money poured into a research programme will have any effect without the one original idea that makes the breakthrough, as has been shown many times in the past.

In our attempts to decide what ideas are worthwhile supporting, we generally rely on the peer assessment system. Groups of successful scientists are asked by government and philanthropic bodies to sit in judgement on the proposals coming forward, to decide which most deserve financial support. This seemingly exemplary democratic system has serious drawbacks, the main one to my mind being the perpetuation of an establishment way of thinking, since the assessment is coloured by the experience of the assessors, who may not recognise the rôle that happy chance had in their own success, nor be prepared to admit that the number of really novel ideas they have had, though brilliantly successful in impact, have nevertheless been few in number. Nor should we forget that Nobel Prizes are sometimes awarded years after the discoveries were made with an approach that was novel at the time but now would seem dated or irrelevant.

The diagram shows a personal view of the characteristic development of research effort in a given area. Programmes stating at different times follow much the same route - a consequence of over-reliance on published papers and accepted methodology, only to arrive sooner or later at the same insuperable barrier, where they remain until some new technology is devised - often in some other scientific discipline, to allow the so-called "breakthrough". The truly original thinker comes in late, grasps the situation, and neatly by-passes the obstacle.



There is an old English proverb - "He who pays the piper calls the tune", and in a democratic society it would not be unreasonable to suppose that the general public who pay both through taxes and by voluntary charity might have some say in what we do. Unfortunately there is often a suspicion almost amounting to fear, and bred on ignorance of what scientists are trying to do. This fear that the search for knowledge may release an uncontrollable monster is clearly stamped on the literature of all ages, from the time Jason planted dragons-teeth, only to be confronted with the unexpected results that sprang forth, and were subdued with difficulty, through Frankenstein, to the wilder realms of present-day "science" fiction about genetic engineering. Second only to fear, as an obstacle to research progress, comes ridicule. How often in the popular press does one meet references to waste of public money on research projects estimated as of dubious value? Yet even here we should be cautious, lest ridicule be turned upon itself.

Jonathan Swift, the 18th century satirist, commented in his book Gulliver's Travels on the absurdity of academic research in his time thus -.

"The first man I saw was of meagre aspect, with sooty hand and face, his hair and beard long, ragged and singed in several places. His clothes, shirt and skin were of the same colour" - (at this point many of us will recognise the description of some contemporary genetic engineer of our acquaintance!).

"He had been eight years upon a project for extracting sunbeams out of cucumbers, which were to be put into vials, hermetically sealed, and let out to warm the air in inclement summers." (Incidentally he was asking for a further eight years support grant.)

Before we agree too heartily with our literary genius' assessment let me draw your attention to the recent Shell-British Petroleum energy award to my colleague Prof. John Pirt.

The invention consists of the controlled growth of

green photosynthetic microorganisms, circulating through batteries of transparent tubing. The sunlight-energy these organisms trap, can be used to produce a host of useful products. Scaled up, this biotechnological device could produce energy at high efficiency, in desert areas, over sea masses and other places that cannot yet be exploited.

I forgive Swift his momentary lapse, for elsewhere in the same book he states -.
"Whoever could make two ears of corn or two blades of grass grow where only one grew before, would deserve better of mankind, and do more essential service to his country than the whole race of politicians put together." Clearly he approved of genetic engineering in the service of agriculture.

Even at intellectual level there is then the possibility of a loss of communication that fosters C.P. Snow's concept of two cultures, though I suspect there are more scientists who have a feeling for the arts than there are artists with an understanding of the physical and particularly the biological world. There is a wealth of inspiration waiting for sculptors and painters in the intricacies of the living cell as seen under the microscope.

The fault lies with ourselves of course, so few of us have the ability to convey what we are trying to do in non-specialised language. In our education system, the concepts of our biological make-up are often taught at a later stage, as though they were excessively difficult, yet one of the first questions a child asks, even before he can read or write, is "where did I come from?" Only much later, in the confusion of adolescence does he ask the philosophical question "where am I going to?"

It occurred to me that an inaugural lecture should do more than recount personal accomplishments in an orgy of self-aggrandisement. It should reveal something of the way the speaker thinks and works, the audience wants to judge not so much how clever he is, but what kind of a creature he

is, and so in attempting to paint my self-portrait in an account of a research programme I hope I have taken that same Cromwell's advice to his painter Lely, to "Paint me, warts and all", for it is the warts, in the form of the shortcomings and dilemmas that give character to the piece.

The first ten years or so of my research career were devoted to studying the metabolic fate of various toxic chemicals, food additives, and drugs. The approach was entirely a chemical one, feeding the substances to an animal in sub-lethal amounts and analysing the excreta to find what modifications had happened during the passage through the body. I became an expert at extracting and purifying unlikely substances from rabbit urine - a somewhat specialised expertise! The detoxification of phenolic compounds is achieved by combining them with a sugar, glucuronic acid, which both moderates the toxic effect and aids rapid excretion, since it is an easily soluble derivative. We also had a passing interest in β-glucuronidase, an enzyme which had the ability to catalyse the breakdown of these compounds, back to the original phenol. Various methods were devised to measure the activity of such a reaction based on the production of light absorbing (coloured) products, from the phenol released. The rabbit played a dual rôle, first as a synthetic chemist, making the glucuronides consistently and efficiently, leaving the biochemist with only the final purification stages to contend with and secondly - post mortem, as a source of the tissues that contained this enzyme; an enzyme whose action was clearly demonstrable but whose function was dimly understood.

In the early 1950's my colleagues were studying the fate of a particular substance called coumarin and noted that the various phenolic derivatives exhibited beautiful fluorescence when looked at under ultraviolet light - a fluorescence that was greatly intensified by making the conditions alkaline. The excreted glucuronide on the other hand would not fluoresce under any circumstances. We recog-

nised that here was a potential new method for measuring glucuronidase by its ability to liberate the fluorescent agent from the non-fluorescent glucuronic acid derivative. Further the degree of sensitivity was greatly expanded. Even with the cumbersome and insensitive methods of detection at our disposal it was easy to detect one thousandth of the enzyme activity that had previously been our lower limit, and the soupy suspensions of tissues we had been forced to use could now be diluted to water transparency and measurements could be made on minute tissue samples.

At this point the leading authority on β-glucuronidase published a review in which he dismissed the method as being interesting but too exacting for general use, a verdict that stood as a deterrent to other users for a considerable time.

Meanwhile in interludes from the Detoxication studies I set about synthesising various other sugar derivatives of the fluorescer - code named 4 M.U. - to demonstrate the general applicability of the method! There was at that time very little evidence in animal tissues for enzymes that attack such substances, and the tests were done on plants, fungi and insects - the resulting publications had understandably a limited readership.

About this time, two important scientific advances caught my interest. The first was the demonstration by Markert and Müller of what were called isoenzymes - physically different forms of what up to then had been thought to be single molecular species, and the biochemical identification by Christian de Duve of a new class of subcellular particles called lysosomes. The importance of this latter was that these particles proved to be the location of the very enzymes I had been working with, and their function now emerged as the means whereby the cell breaks down old unwanted structural material, so that the building bricks can be re-used. This concept of continual destruction and rebuilding of the cell will strike a chord of familiarity to anyone who has experience of the Rotterdam road system!

Thus when the opportunity arose for promotion to a post

in the Science faculty at Queen Elizabeth College, I chose to set up a small group to investigate the nature of multiple forms of lysosomal enzymes. It was I thought a biochemical backwater where I might proceed at my own pace with limited facilities available yet without fear of serious competition.

Shortly after we set up the group at Queen Elizabeth College, Gery Hers, a senior co-worker of De Duve's, published his concept of lysosomal storage diseases as enzyme defects in the degradative pathway. These rare diseases had puzzled medical scientists for a long time. The symptoms had been well described and in most cases there was clearly a hereditary link, and the condition was apparently recessive - affected patients often having perfectly normal brothers and sisters. Typical symptoms were stunted and deformed growth, mental retardation and a general inability to thrive, so that it was not uncommon for the patient to die in early infancy or childhood of some infection. Microscopic examination of the tissues after death frequently showed an abnormal accumulation of material stored in the cells, we now know it is stored in the lysosomes, and a chemical analysis of such materials indicated that this varied according to the particular syndrome being examined. Complex carbohydrates constituted the major part of such storage materials.

The explanation of the defect is quite simple, these complex molecules consist of long strings of a variety of different sugars and other molecules. They can only be broken down for re-use, by starting at one end removing the components one at a time. A different enzyme is required for each sugar and to decompose the complex structure completely, a whole range of enzymes (all found in the lysosomes) must be able to work in the correct sequence. If any one of these enzymes is defective the process will stop at that point, leaving the partly degraded cellular rubbish to accumulate. The tissues become an out of control garbage dump. The concept can be confirmed, since the terminal sugar on any accumulated material ought to be that one for which there is

a faulty enzyme. Thus the disease could be diagnosed either by a careful chemical analysis of the accumulated material or by detecting the absence of an enzyme that is normally present. Only in recent years has the reliability of structural chemical analysis made the former possibility feasible. On the other hand, with the new fluorescent substrates, it was possible to make up a balance sheet of the amount of many lysosomal enzymes we would expect to find in various tissues, and with this information look for any debits in the form of missing enzymes.

The methods worked well to identify the enzyme deficiencies in a number of diseases, and the hereditary nature could be confirmed by studying the enzyme levels in the parents in many cases. With some notable exceptions, the appropriate laws of heredity were those discovered by Gregor Mendel while growing peas in a monastery garden at Altbrünn in the 1860's.

The parents while physically unaffected, should have roughly half the levels of the enzyme that we might expect to find in a normal population. About half their offspring should be like themselves, so-called carriers, one quarter should be free of any defect while the other unfortunate quarter would suffer from the disease because of a lack of the enzyme.

The methods of diagnosis, which had previously relied on a superficial physical examination of the patients' disabilities, now moved into the greater precision of the diagnostic laboratory. Living cells grown from small patches of skin from the patients could be examined and distributed to other laboratories around the world for collaborative studies. The methods were refined to a high degree of sophistication, notably in the Dept. of Cell Biology in this University, to the point at which a prenatal diagnosis could be made on an unborn child by testing the cells in the fluid surrounding it in the womb. There was growing confidence in biochemical analysis and pre-natal diagnosis.

Unfortunately this approach has the one great dis-

advantage in that an affected child has to be born before we are aware that two parents are carriers of the same gene mutation. How much better it would be if we could detect this carrier status before reproduction, then even the birth of a first defective child could be prevented.

This approach to carrier screening became possible in the case of the lysosomal storage disease Tay-Sachs disease. In the 1890's Warren Tay, an ophthalmologist, had described an unusual eye condition in a retarded child. Simultaneously a neurologist named Sachs described a disorder in an unrelated case that eventually appeared to be the same syndrome, severe malfunction of the brain and nervous system being the main symptoms. This disease, although still of rare occurrence, was distinctly prevalent in the Ashkenazi jewish population in which the gene seemed to have been perpetuated at an unusually high level. By mid 1960, chemical analysis of the storage material would suggest that the enzyme Hexosaminidase was the putative mutation point. Apart from an isolated report by Sandhoff of the expected total absence of hexosaminidase in the tissues of a deceased patient, other workers found if anything elevated amounts of enzyme activity in their pathological samples.

It happened that at this time we were examining the isoenzyme possibilities of hexosaminidase and found it to consist of two forms, a rather acidic form A, and a more basic form B, easily separated from each other by conventional laboratory procedures, such as gel electrophoresis, in which the components are separated from each other in a slab of starch gelly by applying an electrical potential across it. Because the components have differing degrees of electrical charge, they are drawn through the gel at different speeds and soon become spaced apart. We found they were also distinguishable by the fact that the A form was readily destroyed by gentle heat under conditions where the B form was unaffected.

We could measure the relative amounts of each present in a mixture by first measuring the total, then heating to

destroy the A form and measuring the B remaining. Similar amounts of each normally occurred in most normal tissues.

This simple device was taken up by the paediatric biochemists on a re-evaluation of their findings. The Tay-Sachs samples were now shown to be devoid of the heat-sensitive A form, but had compensating amounts of B. Thus while the level of activity was misleading the absence of a specific molecular form was irrefutable. Parents of such children could now be shown to have about half the usual levels of Hexosaminidase A but interestingly enough, Sandhoff's samples were reconfirmed as being devoid of all activity, and further patients usually of non-jewish origin were being detected with similar results.

The outcome of a long and still ongoing investigation of the molecular make-up of these enzymes has resulted in the consensus that two independently genetically controlled components are involved. One of these proteins, the B sub-unit, is common to all forms of the enzyme and when it is defective, no activity at all can be expressed, as in Sandhoff's patient. The other is a component peculiar to the A form of the enzyme and imparting to it essential properties for its in vivo function. When this A sub-unit is affected, the A enzyme is inactive, but B forms can function normally.

While both A and B forms act upon our test substrates equally well, clearly each has an essential biological function to perform, since a lack of the A form results in such severe physical and mental handicap in Tay-Sachs disease. Limitation, if not elimination of Tay-Sachs disease offers a model of what can be done in the future. Demonstration of hexosaminidase A deficiency has become relatively easy and inexpensive. It can be carried out on a number of easily accessible sources of human material, blood, tears, skin cells, by automated procedures. Both patients and healthy carriers can be detected.

Once such a methodology becomes available, the question arises what sections of the population should be selected for a screening programme, since obviously it would be

impossibly uneconomic to attempt to test every single individual in such cases where the frequency of carriers is very low.

We know, however, from population studies, that in jews of Askenazi origin about 1 in 20 is a carrier of the mutant gene as compared to 1 in 300 in the general population. This then is a selected population at high risk. Nevertheless, mass screening programmes only became possible after a long period of public education to the risk through newspapers, TV, etc., and after discussions with the administrative authorities who would have to organise such tests. In this respect the religious leaders played an important part. Attempts to provide such a service in London largely failed as a result of a much weaker response to Rabbinical advice than elsewhere.

During the ten years following the development of the methodology over 312,000 people were tested in 13 different countries. Nearly 13,000 carriers have been identified and 268 couples had been shown to be at risk at the latest count.

In the absence of a treatment, those couples were now faced with several options. They could of course decide to reproduce and accept the 1 in 4 risk that their child would be affected. At the other extreme they could decide to remain childless. Or they could request pre-natal monitoring on the state of the foetus and elect for abortion, if the tests prove positive for Tay-Sachs disease. Out of 814 pregnancies, 175 cases of Tay-Sachs disease have been diagnosed before birth, and it has been calculated that the incidence of children born with the disease in North America has been reduced by 65-85%.

What I have described is just one of a large number of rare inherited diseases in which we can now diagnose not only the defect in a patient, but also the potential of a parent to produce such a child. In each of these cases the procedure has been much the same - to identify what component of the normal system is missing, and then to develop suitably

sensitive tests to confirm its presence or absence in cells and tissue samples.

Such methods pre-suppose that we have a considerable knowledge of the make-up and function of the enzyme concerned, but there are many instances where we know of an obvious defect and its genetic transmission without being able to define the precise agent responsible. If a machine does not work, and we are familiar with its working, it is not too difficult to find out if a piece is missing. If we are not so well informed about the machine, we may still be able to diagnose a fault, if we can read the instructions by which it was constructed.

The recent developments in Genetics involving so-called Recombinant DNA technology hold out an enormous potential for doing just that, and for noting when the instructions contain an error or omission. The advantage here is that we may be able to detect an omission in the instructions, even if they are written in a language we don't fully understand, and by relating the position of the error in the text, with the observed malfunction, we have a means of diagnosis. In order to do this the instructions have first to be extracted from the mass of genetic information that exists in the cell, copies of these instructions have to be made in sufficient quantity for us to be able to see them by the limited methods at our disposal, and they then have to be cut up into short sentences or paragraphs of information of a handlable size. We can then compare the various sized pieces from a normal set of instructions and from a set from an affected individual and look for where differences occur. The parent-carriers have both sets of instructions in their genes and so the error fragment will also be found there.

Already such a direct recognition of an error in the genetic instructions has been applied to the detection of certain hereditary anaemias that are widespread through the Mediterranean, Near East and the African continent. In this case the work has been made possible by the previous detailed knowledge of the chemical structure of the blood protein

involved, so that it was possible as it were, to find the right page where the instructions were.

Without such knowledge, the task becomes akin to finding an error in the Encyclopaedia Brittanica, but as methods for extracting genes and magnifying them improve, and with the use of automated and computerised analysis, we may foresee our eventual ability to read the whole book of life which is the genome.

All the tools, monoclonal antibodies, translation systems, copying systems and visualising techniques have been developed over the last few years by the molecular biologists, by ingeniously exploiting the natural functions of living cells, particularly microbes and turning them to work for us. There was initially considerable apprehension at the notional hazards of these procedures. Such exploitation of biological functions has of course been a distinguishing feature of intelligent life through the ages, and what the molecular biologists are doing here is in a higher degree of sophistication no more than what the Dutch farmer does when he utilises the annoying bacterium that turns his milk sour into a useful agent for producing cheese. Far from fearing these advances, we should embrace them gratefully and put them to the service of mankind.

It is essential that the information programme in these diagnostic services is carefully and sympathetically managed. Carriers may well develop feelings of inadequacy or guilt at producing a handicapped child. Marital conflict could arise, particularly when the defect is associated with the female chromosome, the so-called X-linked diseases such as haemophilia and Duchenne muscular dystrophy, which only affects male offspring of a carrier mother who becomes to feel somehow it is her fault - "the sins of the mothers being visited upon the children, even unto the third and fourth generation" in a biblical paraphrase. Nor should there be any suggestion of racial unequality in this respect. Each ethnic group has its own problems - Tay-Sachs disease in jews, blood disorders in negroes and asians, cystic fibrosis in caucasians. Indeed

genetic research indicates that each of us carries at least 2 or 3 lethal gene mutations, and all of us are carriers protected only by the fact that the chance of our chosen mate having the same defects is infinitely small.

In this field ethical discussions tend to be charged with a great deal of emotion but somewhat lacking in information and understanding. It is the duty of those of us who generate that knowledge to see that it is disseminated widely. Nevertheless, there remain troubling ethical problems that will continue to arise as more and improved methods for carrier detection and early diagnosis of these diseases become available.

Situations arise where we can diagnose at birth a disease that cannot be treated, and that will only become apparent some years later, as for instance Duchenne muscular dystophy. Screening is perfectly feasible but is the knowledge desirable? If we do not tell the parents, they may produce several more affected sons before the eldest shows symptoms. If we do tell them, how can we place on them the burden of trying to bring up their child in a normal and happy fashion, knowing what fate has so shortly in store. How is a mother going to feel in telling her daughters that they too are carriers. Yet if we do not tell we may be bitterly resented for not forewarning the need for making financial preparations early, e.g. for buying a house suitable for the disabled to live in.

There are many young people who are now approaching marriageable age and who have this knowledge of a defect in their own genetic make-up. A decade ago most of the people requesting genetic advice were parents who already had had an affected child. Today the majority of requests for genetic counselling come from young people who have not yet reproduced but who fear an increased genetic risk. All at once our expertise burdens us with an influence on the decisions that surely should be taken primarily on emotional grounds, love, affection and compatibility, yet the influence of an affected child on a young couple without the buffering

protection of the old style extended family could equally eat away at those finer feelings. A recent study of the divorce rate in families with a spina bifida child showed that this was nine times higher than in a comparative population.

There is a body of expert opinion who would advise avoidance of this dilemma, preferring to wait until a pregnancy occurs before making the tests in the course of the normal pre-natal care. At the same time, the options now become limited. If the test is positive, the husband must be tested. If his test is positive, the pregnancy must be monitored, and in the case of an affected foetus, the pregnancy can be terminated, the only option remaining.

In the final analysis, the decisions of how far mass screening procedures are introduced into medical care will be taken on political, that is to say economic grounds. The ensuing benefits to future generations do not weigh so heavily with politicians as do ventures with more immediate cost/benefit appeal. Yet it is not difficult to find ample evidence that even in the rare diseases I have described, screening offers a hard financial advantage. Added to this, the fact that most of these patients die at a very early age means that the number of useful life-years lost to the community is far greater than those lost through the more common causes of death which have their impact in our declining years.

In Rotterdam alone, some 5000 or so pregnancies have been monitored and the birth of about 250 severely handicapped children has been prevented. Many of these would have died at an early age - others might have lived much longer but with increasing severe physical and mental handicap. If each had survived an average 10 years the total cost of their care would not have been far short of 250 million guilders. The cost of the prenatal program, both diagnostic and counselling, is of the order of 2.5 million, or 1%.

Or again, about 1 in every 4000 boy babies is born with

Duchenne muscular dystrophy. About 80,000 boys are born in Holland every year. It would cost about 5 guilders to test each of them, a total of 400,000 fl. The cost of care of one muscular dystrophy patient has been calculated at around a minimum of fl. 250,000. Thus if as a result of a post-natal screening, the birth of 2 such children were prevented by parents refraining from having further male children, the costs would be justified. If as a result of carrier detection and prenatal tests, the number of such births could be halved from the present 20 or so a year by genetic counselling or therapeutic abortion, the savings would be proportionally greater: Cold calculations for a tragic human problem, not helped when set against the choice of spending 1 million guilders or a heart transplant, or spending the same sum to feed starving children in the 3rd world.

Our hope for the future is in developing a therapy and in a few cases this is already possible. Sadly many of the diseases I have talked about do not offer such an easy solution. We need more basic research into the precise nature of the diseases. We need to understand more of the biochemical processes in which the defective agents are involved, and we need to develop new and ingenious ways of setting them to rights. No one can predict the route of enquiry that is most likely to lead to such findings. No one can tell just when and where the happy accident will occur. As research biochemists and geneticists we can only hope that when our work is evaluated it will be recognised, that our academic freedom is indeed in willing bondage to social responsibility.