PART 2

ADDRESSES AND CONTRIBUTED PAPERS

Notre Dame, Indiana
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PRESIDENTIAL ADDRESS

The address, "Pharmaceutical Research: Its Contributions to Science and Medicine," was presented by retiring president, Dr. Otto K. Behrens, Eli Lilly and Company, Indianapolis, Indiana 46206, at the annual Fall Meeting dinner at the Saint Mary's College Dining Hall, Saint Mary's College, Notre Dame, Indiana, on Friday, November 3, 1972.

SPEAKER-OF-THE-YEAR ADDRESS

The address, "The P's and Q's of Modern Astronomy," was presented by the Speaker of the Year for 1972-73, Dr. Frank K. Edmondson, Department of Astronomy, Indiana University, Bloomington, Indiana 47401, at the annual Fall Meeting luncheon at the Saint Mary's College Dining Hall, Saint Mary's College, Notre Dame, Indiana, on Friday, November 3, 1972.

PRESIDENTIAL ADDRESS

Pharmaceutical Research: Its Contributions to Science and Medicine

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The attitude and understanding of the nature of industrial research is obviously of importance in the organizations in which it is being conducted. Perhaps less obviously, industrial research also should be understood in the academic community, and indeed by our society. If it isn't, our society will be denied the optimal contributions that can be forthcoming from industry, and solutions to many of our problems will be delayed or may be made impossible. As I have been a part of the research establishment of one of the major pharmaceutical companies for more than 30 years, let me make some observations intended to be informative and that may, at the same time, be interesting.

Over the years, I have been intrigued with the variety of viewpoints toward pharmaceutical research encountered among my academic friends. These have ranged the gamut from the condescending or sometimes distrustful, through attitudes of mystery or ignorance, and extending to appreciation and even glamour. Perhaps the most common is one of some condescension, implying that pure research is the only career worthy of the superior mind and that making science useful is a less worthy objective. At the same time, we all recognize that the tremendous support of science during recent decades developed because science and technology supplied products that people wanted and needed. As stated by Dr. Robert B. Carlin, Professor of Chemistry, Carnegie-Mellon University (2):

> "Chemistry has attained its eminent position among the sciences by being useful. A large, stable chemical industry continues to produce the myriad of useful items that chemical science and technology have provided. Had chemistry failed to supply materials that people want and need, professors of chemistry would now be as numerous and influential as professors of Greek, and there would be no chemical engineering.
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> "In the face of these obvious facts, too many college and university chemistry faculty members teach chemistry to their students as though it were Greek. The subject is presented as a fascinating intellectual exercise (which it is), to be pursued for the stimulation it affords the intellect, like chess. Implicitly or explicitly the student is given the impression that making chemistry useful is somehow degrading. By example, if not by word, he is taught that pure research is the only career in chemistry worthy of the superior mind.

> "College and university programs in chemistry have reflected this point of view. Undergraduate training now is designed to prepare the student for graduate school; graduate education prepares him for an academic career. We behave as though there are no alternatives. A graduate who accepts a position in any professional endeavor other than pure research is likely to feel that he has demeaned himself."

Dr. Carlin was speaking to chemists. Otherwise he might have expressed the same viewpoint about a number of other sciences, *e.g.*, endocrinology, pharmacology, microbiology, etc.

Now, permit me to recount some of the experiences that I have seen or in which I have participated. Perhaps we should start by taking cognizance of terms such as basic and applied research or academic and industrial research. These terms imply a definite difference and may well underlie the condescending viewpoint. One differentiation between the two has been based on a definition of basic research as directed toward seeking knowledge in order to understand the basic nature of the universe. In contrast, applied research has been defined in terms of solving a problem, of making a contribution to human welfare. Gradually, the realization has grown that the definitions are not mutually exclusive and indeed may be inadequate and misleading. Repeatedly through the years, new knowledge concerning the laws of nature has opened doors to new practical applications. The importance of basic research in this respect is so well recognized that I need not

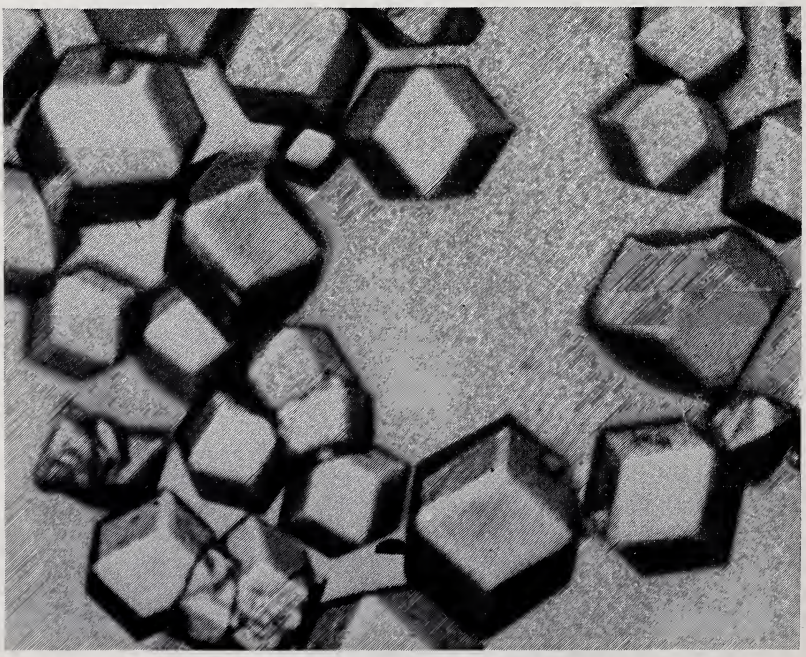


FIGURE 1. *Glucagon Crystals.*

document the statement. However, inadequate emphasis has been placed on the converse, that research aimed toward solution of problems leads to new insights into the nature of the universe. Much of my discussion will be directed toward this latter point.

During the early 1950's, I was a part of a research project that is still having ramifications in basic as well as applied research. When Scott discovered a new practical procedure for crystallization of insulin in 1934, the crystals were found to possess the usual hypoglycemic properties useful in the treatment of diabetic patients. However, for a few minutes after administration, a hyperglycemic response was observed. Was this opposite type of response due to insulin? Or did it indicate the presence of an impurity, and if so, was its presence undesirable? As a leading supplier of insulin, we felt a responsibility to understand the situation.

About 25 years before we started our work, Murlin (5) in 1924 had published evidence for the presence of a hyperglycemic factor in the pancreas and had named it glucagon. Working with side fractions of the insulin purification, we were successful in concentrating and purifying the hyperglycemic principle and, in 1953, we announced the isolation of the new hormone in crystalline form (8) (Fig. 1). We retained the name, glucagon, suggested by Murlin. With the pure material in hand, work was possible to determine its physical characteristics and to explore its biological significance. Very quickly, we determined that glucagon represented a large polypeptide or small protein containing 29 amino acid residues. The amino acid sequence of insulin had just been determined by Sanger, and we recognized that similar work should be done with glucagon as a contribution to the understanding of protein structure. Within a short time, we determined and published the amino acid sequence (1) (Fig. 2).

AMINO ACID SEQUENCE OF PORCINE (BOVINE) GLUCAGON

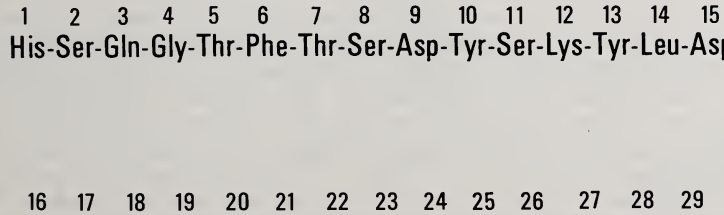


FIGURE 2. *Amino Acid Sequence of Porcine (Bovine) Glucagon.*

At the same time, our clinicians determined the usefulness of glucagon injection in the diabetic who had overdosed himself with insulin and was experiencing an insulin-reaction. Samples of glucagon

also were made available upon request to scientists interested in the significance of the hormone in the general economy of the body. Such work still continues. As a result of such studies by our colleagues at Lilly as well as by scientists in other laboratories, the clinical usefulness of glucagon is presently being explored in treatment of certain heart conditions and as a diagnostic agent for some gastrointestinal conditions.

Although we had concluded that the small glucagon impurity in insulin was unlikely to be of significance in the treatment of diabetes, newer methods of purificiation have now led to commercial insulin substantially free of glucagon.

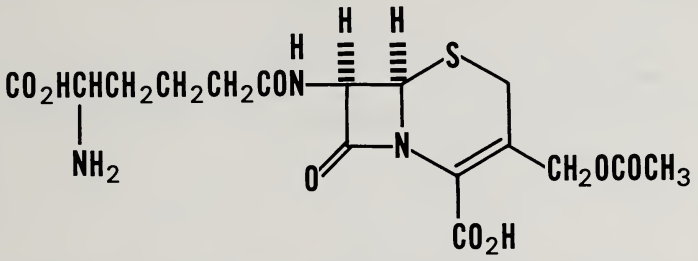
On the basis of this brief summary, you may agree with me that our research on glucagon, contributed to basic knowledge as well as to clinical applications.

Turning from glucagon, I'm confident that you are fully aware of the practical importance of making medicines available in an economical way. Numerous examples may be cited of exorbitant early costs that were brought under control by so-called developmental research. Indeed, such work is often considered a classic example of applied research. Let me cite an example to indicate the difficulties in classification.

The story of the discovery and development of the important cephalosporin antibiotics is one of the adventure stories of modern medicine. It started in 1945 with the isolation of a fungus that produced antibiotic activity by an Italian professor, Giuseppe Brotzu. He reported a number of interesting characteristics but concluded that the isolation of the active principle would be beyond his resources. In 1948, an active investigation was undertaken by the brilliant group at Oxford University, which included Lord Florey (then Sir Howard), Professor E. P. Abraham, and the late Dr. G. G. F. Newton. After careful work they isolated an antibiotic, cephalosporin N, which proved to be a true penicillin and was renamed penicillin N. In 1954 they detected the presence of small amounts of another antibiotic. Within a year they had reported the isolation and characteristics of this compound which they called cephalosporin C. Although the specific activity of the antibiotic was quite low, it had several characteristics that were a powerful stimulus to further investigation. It had a broad range of antibacterial activity, it killed bacteria quickly, its toxicity to mice was negligible, and it was resistant to hydrolysis by penicillinase, the enzyme that destroyed classical penicillins. Furthermore, none of the strains of antibiotic-resistant organisms against which it was tested were resistant to cephalosporin C.

By 1960 two groups of investigators, Professor Abraham and Professor Newton and Professor Dorothy C. Hodgkin and Dr. E. N. Maslen, were able to publish the complete structure. The molecule may be considered as a nucleus bearing some resemblance to the penicillin nucleus, and two side chains, an acetyl group, and an amino-adipyl group (Fig. 3). The Oxford group set out to study the replacement of side chains to determine their effects on activity. Efforts to remove

or displace the amino adipic side chain were seriously hampered by the relative instability of the remaining nucleus, called 7-aminocephalosporanic acid, or 7-ACA. Nevertheless, using extremely fine methods of detection and isolation, the Oxford group demonstrated the presence of small amounts (<1%) of 7-ACA following hydrolysis of cephalosporin C in dilute acid. Reacylation with various side groups led to new substances, some of which had markedly enhanced antibacterial properties. For example, the phenylacetyl derivative had an activity against staphylococci that was several hundred times that of cephalosporin C. Quite obviously, major problems needed to be solved if these interesting cephalosporin antibiotics were to play a role in human medicine.



E.P. Abraham and G.G.F. Newton, Biochem. J. 79, 377 (1961)
D. Hodgkin and E.N. Maslen, Biochem. J. 79, 393 (1961)

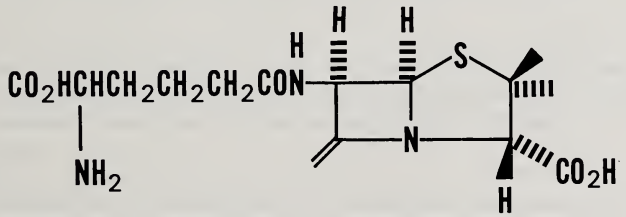


FIGURE 3. *Cephalosporin C (upper structure) and Penicillin N (lower structure).*

One of these problems consisted in the removal of the aminoadipyl group in good yield. Work in several laboratories, including our own, to find a solution by various hydrolytic procedures or by microbiological conversion came to naught. Fortunately, some papers had appeared on facile cleavage of amide bonds by reactions that involve neighboring group participation. Whether such cleavage reaction would be applicable to the cephalosporin molecule and the reagents needed to bring it about were quite uncertain. Nevertheless, members of our organic staff decided to attempt such a cleavage (7). When cephalosporin C was treated with aqueous nitrous acid, two molar equivalents of nitrogen were produced, but very little nucleus was recovered. Dr. Robert B. Morin deduced that in aqueous solution the intermediate iminolactone was being hydrolyzed. Use of nonaqueous solvents might avoid this destructive reaction. Indeed, when acetic acid was used with

nitrosyl chloride and the nitrosating agent was removed before contact with water, a 7% yield of 7-ACA resulted (Fig. 4). A study of reaction conditions led to replacement of acetic acid by formic acid and increased the yield to 25-40%. Thus, a significant extension of new chemistry was an essential element in solving a problem in drug preparation, and in recent years, the cephalosporin antibiotics have become a major addition in the continuing fight against infections.

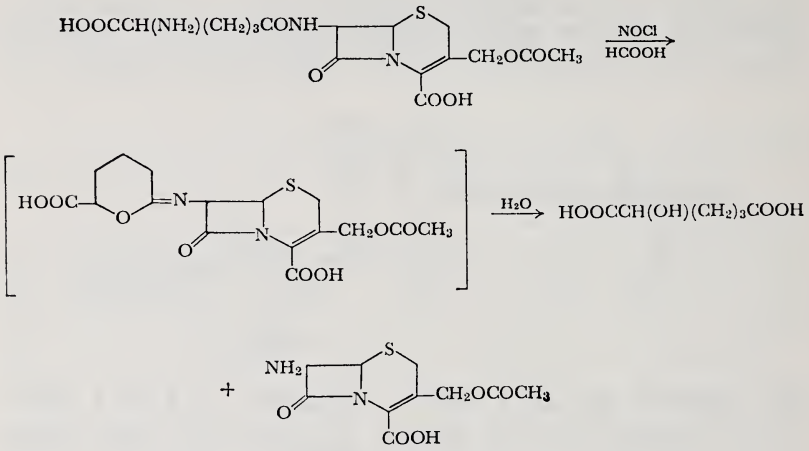


FIGURE 4. *Cleavage of Cephalosporin C to 7-ACA.*

The discovery and development of new medicinal agents also serves basic science in another manner. These substances frequently serve as new tools for the study and elucidation of the essential metabolic processes of life. Many of you are aware of the elegant studies by Strominger and collaborators in which he used penicillin as a tool to elucidate basic processes involved in the formation of some constituents of cell walls. With the development of the cephalosporins, which are substances with significant similarities as well as marked differences from the penicillins, a new related tool became available. The cephalosporins have served to confirm and refine the studies on mechanism of synthesis of cell wall constituents. They are also serving an important role in comparative studies with penicillins on the mechanism of antibacterial action.

Appropriately, we should examine another type of activity that is considered by many as characteristic of the search for new drugs, the screening of candidate compounds for desirable types of biological activities. Perhaps a great many scientists would characterize such research as dull and comparatively unrewarding. Indeed, if a given screening test system is set up and is used for a prolonged period of time, it does become routine. However, a prolonged reliance on a given screening test imposes certain limitations that may not be apparent to many individuals. A given simple test system that is suitable for screening large numbers of drugs may be characterized through its

potential to detect compounds that possess a desired activity. In most instances this activity will be manifested through one or at most a very few mechanisms (*e.g.*, estrogenic, anticholinesterase, etc). After a compound is found that possesses the desired activity and that is relatively non-toxic, further use of the screen will most likely lead to "me-too" drugs of diminishing return for the medical profession as well as for the pharmaceutical company. Obviously then, excessive routine screening is not good pharmaceutical research. Then how should we proceed? Perhaps the following example will afford some insights.

Several years ago we became interested in a problem that afflicts a sizeable proportion of our population, obesity. Other investigators had demonstrated that the hypothalamus plays a role in affecting food consumption. Within our research function, the question was posed whether characterization of this role might lead to new approaches for the control of appetite. Dr. Paul Stark and associates have shown that a cholinergic system in the hypothalamus decreases the threshold for electrical stimulation of appetite (9). He has proposed "stop" and "start" systems for eating that are adrenergic and cholinergic, respectively (10). Activation of the "stop" system by the use of adrenergic agents such as amphetamine has been used clinically for many years to suppress appetite. Dr. Stark suggested that the possibility of using specific anticholinergic drugs to suppress the cholinergic "start" system is an equally logical approach to appetite suppression.

Quite obviously, this work has led to a new approach to the screening and selection of candidate compounds. It illustrates that good science is needed in the process of developing screening procedures. Furthermore, the above example is but one of a number that might have been selected. It illustrates the interrelationship of new knowledge of life processes to the efforts to contribute to solution of medical problems.

A number of other kinds of scientific endeavor are found among the research and development activities at Lilly, such as toxicology, analytical chemistry, physical chemistry, medical research, fermentation research, microbiology, virology, plant physiology and pathology, animal nutrition, etc. In all of these fields, the pursuit of the application of knowledge to the solution of problems and the development of products is accompanied by contributions to the basic understanding of the universe.

As one additional example, I will cite some research in drug metabolism. Studies in drug metabolism have come to be considered essential as a part of the understanding of drug action. How a drug is absorbed, its concentration in body fluids and tissues, its effect on enzyme systems, its metabolic transformation in the body, and the manner and extent of its elimination are all important elements that permit pertinent comparison of the effect of drugs in animals and man. Such studies are of importance in understanding the manner and duration of drug action, both with respect to desired activities and with respect to undesirable or toxic manifestations.

In early studies on the metabolism of the hypoglycemic agent, acetohexamide, Welles, Rost and Anderson (11) found that the major route of metabolism is to hydroxyhexamide (Fig. 5). Subsequent synthesis and testing of dl-hydroxyhexamide showed that it is as active as acetohexamide. Studies on the blood levels following administration of acetohexamide or hydroxyhexamide to diabetic patients indicated that the half-life of acetohexamide is 1.6 hours, whereas the comparable figure for the hydroxy compound is 4 to 6 hours. Thus, the conversion of approximately 80% of acetohexamide to hydroxyhexamide, an active compound, and the slower excretion of the latter compound represent major factors in understanding the extent and persistence of the blood sugar lowering activity.

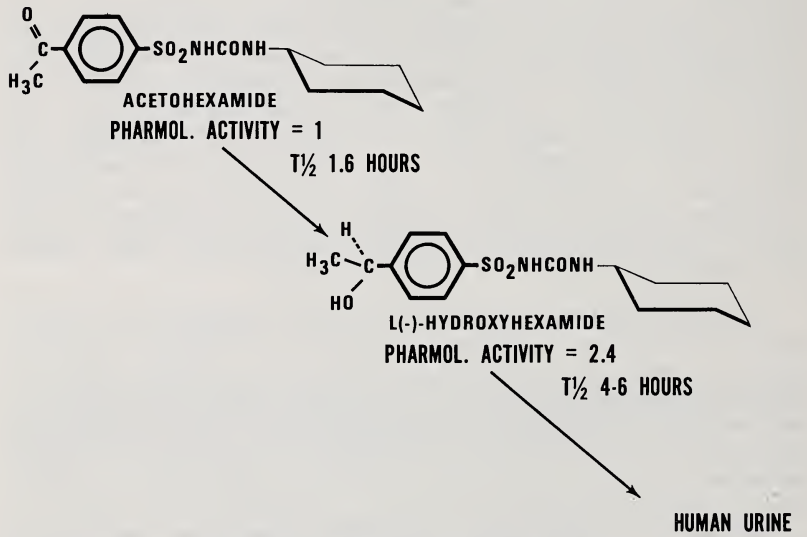


FIGURE 5. *Metabolism of Acetohexamide.*

This is not the end of the story. McMahon and associates determined that the hydroxyhexamide produced in the body has the L-configuration and that this isomer is about 2.4 times as active as acetohexamide (6). As a consequence of these findings, Culp and McMahon (3) undertook a study of the enzyme responsible for this reduction. They partially purified an enzyme, aromatic aldehyde-ketone reductase, from kidney cortex, and found that it is capable of reducing a variety of aromatic aldehydes and ketones and that it utilizes the β-hydrogen atom of C-4 of TPNH. Subsequently, R. B. Hermann and associates published a study on the substituent effects among substituted phenacyl derivatives employed as substrates for this enzyme (4). The physiological significance of this enzyme has not been established. However, investigators in other laboratories have prepared the "Culp enzyme", and undoubtedly its role in the metabolic economy of the body will be determined.

Before drawing some conclusions, I should emphasize that many more examples are available within our laboratories of contributions to basic understanding of matter and of life that were made during the pursuit of practical goals. If, then, the boundaries between applied and basic research are not sharp and well-differentiated, how else should we characterize the research performed in our laboratories? In a recent conversation with one of my colleagues, he suggested that he had decided simply to rate all research, industrial or academic, on a scale ranging from excellent research to poor research. Then he said that he has realized that good or excellent research is found in both industrial and academic settings, and, unfortunately, the same is true for poor research.

Perhaps the most significant difference between academic and applied research is that of motive. Applied research seeks to make available products that are needed by man. Indeed, one of the major attractions of industrial research for many scientists is the desire to bring good science to bear on the solutions of problems. The opportunity to make a direct contribution to the welfare of man through the discovery and development of useful products, in my opinion, provides a worthy challenge.

At the same time, imaginative applied research offers major opportunities for obtaining knowledge and understanding of the basic phenomena of our world. These opportunities often arise as a direct necessity of the research itself, and might not have arisen out of academic or basic research. Applied research, then, constitutes a significant and separate approach to the more adequate understanding of our world. Furthermore, the findings and products of applied research frequently supply new tools and approaches to the academic scientist.

In my viewpoint, these challenges and opportunities of industrial research need no apology. To meet them adequately will require greater understanding, and a change in the teacher's or professor's viewpoint of the occupations that are worthy of our best minds. By word and example, students need to be shown science being used for the welfare of mankind. For me, this occurred outstandingly during a freshman course in chemistry at DePauw University. Repeatedly, Professor W. M. Blanchard cited examples of practical applications of chemistry as well as challenges for the future. These insights were of major importance to me. As I sought employment, I looked for opportunities to pursue sound scientific endeavor, irrespective of the sponsoring organization. In my opinion, the continuing challenges through the years have fully justified this approach!

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