PIECES IN THE PUZZLE

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I suppose the main reason for the Editors to honor me by asking me to write a Prefatory Chapter for the Annual Review of Pharmacology is that my activities seem to have dealt more with pharmacology than my official title as professor of Physiology might indicate. If so, this is no doubt due to the fact that some of my most prominent teachers have been professors of pharmacology or served as directors of pharmacological laboratories—G. Liljestrand, H. H. Dale, and C. Heymans—although I would still regard them as essentially physiologists. However, their knowledge of pharmacology was profound and it is not surprising that their familiarity with pharmacological tools has been to some extent transferred to their pupils. Dale introduced the term "autopharmacology," signifying the actions of certain substances occurring naturally in the body ("Körpereigene" in German) which in some respects resemble pharmacologically active drugs. At least the description and the analysis of their action often followed patterns similar to those applicable to various drugs used for pharmacotherapeutical purposes or as tools in the attempts to classify and understand the action of other drugs.

After the end of World War I experimental physiology and pharmacology expanded rapidly. O. Loewi and W. B. Cannon gave the long wanted definitive proof of chemical neurotransmission, which was to have such far-reaching consequences. Acetylcholine, chemically synthesized many years previously and studied by Reid Hunt and Taveau in 1906, had caught the interest of many research workers including H. H. Dale, who soon noticed the potential importance of this compound for biology. Histamine also came into the picture, and was even implicated in the cause of wound shock during the 1914–1918 war. Several other "biogenic amines" were isolated during this period and their actions studied.

The conditions for scientific work in the medical field in Scandinavia after World War I could hardly be characterized as very favorable as regards localities, laboratory equipment, personnel, and funds. Many of those who still found research work attractive, in spite of the few positions available and the modest salaries, were undoubtedly beset by the same peculiar urge to find out what the mechanisms were behind the biological phenomena with which they had been vaguely acquainted in the preclinical studies. Intellectual curiosity and a desire to "explain" various phenomena are probably still the most effective forces driving the young student into an uncertain future in research. The motivation may of course be more complex, and in addition to the hope of making useful discoveries, a pleasant feeling of becoming a member of what appeared to be a distinguished fraternity might also enter. An incentive of a rather special kind was sometimes offered by the early discovery that some explanations in the textbooks did not appear to be too convincing. This was almost an invitation to provide the correct answer as a result of clever experiments and new approaches which presumably had not entered the mind of the textbook writer. This included no doubt a touch of emulation which may not be considered as very distinguished in research, but nevertheless can have an activating effect. In spite of certain hardships, those who started research work in the twenties were in a privileged positions, or at least this was my own feeling. The research climate was improving and we were aware of an upsurging interest which to a large extent was to be credited to a local group of young scientists of Karolinska Institutet.

The genetic factor must of course not be overlooked. In my own case such factors may have been inherited both from my father, who was a biochemist, and my mother who began with botany and specialized in diatomes, following up the work of her father, who was also professor of inorganic chemistry in Uppsala and discovered the elements Scandium and Thulium. It is probably quite common that a son of scientists at an early stage becomes engaged in some kind of research work. Through my father's wise suggestion I received an excellent introduction into research by joining the group around Robin Fåhreaus in 1925. Fåhreaus, who had then already won considerable fame by his discovery of the sedimentation reaction in the blood, had a singular gift of enthusing his disciples, spurring their curiosity and interest. Every observation was "important" and could lead to results of great biological significance. Fåhreaus had all the charm of a gifted scientist who could provide the "ignition" impulse to start one's mind in the research direction. Equally helpful was my teacher Göran Liljestrand, who set off the second stage, in the form of a Rockefeller Fellowship, allowing the happy holder to study abroad with outstanding scientists. One can only wish that today's young research workers could experience the same happiness as befell one when the letter of acceptance arrived. I have since learned that many of my colleagues agree with me that these Fellowships have been truly instrumental in creating the solid basis for a research career, including a certain status.

Also here the choice suggested by my teacher was excellent; I was to study for half a year at The National Institute for Medical Research in London under H. H. Dale. At this time, 1930, rapid progress was made in experimental pharmacology and physiology, and Dale was one of the most renowned and successful leaders in this field. Dale combined precision, careful experimenting, and critical evaluation of the results with thorough knowledge of the literature and scientific phantasy. His laboratory was an ideal place for a young scientist with open eyes and a willingness to learn.

Acetylcholine was in the center of interest in many laboratories and Dale must have felt that it played a central role in physiology. The intense action of this drug on the motility of the gut suggested that it might serve as an intestinal motility hormone, or "Hormon der Darmbewegung," as previously ascribed to choline by le Heux, who based this concept on the results obtained with drugs on the isolated intestine as used in R. Magnus' laboratory in Utrecht.

My first task in Dale's laboratory was to try to demonstrate the presence of such a hormone in the effluent of a perfused intestine of the turtle and rabbit after nerve stimulation. This failing, I resorted to the simpler task of searching for it in extracts of rabbit intestine. The result appeared most encouraging when the extracts were tested on an isolated piece of rabbit jejunum, since this contracted very nicely on addition of the extract to the bath. However, addition of atropine to the bath fluid did not suppress this effect, which seemed then to exclude choline and acetylcholine, and with youthful enthusiasm I declared that a new biologically active substance had been discovered! This was of course not immediately accepted, but at Dale's suggestion the effects observed became subject to further study, in which I had the privilege of working with John H. Gaddum, then first assistant in Dale's laboratory. After some months of hard work and valuable advice from the experienced chemists at the Hampstead Institute it became reasonably certain that the active factor was a new active principle, which was simply called "P" (for our standard Preparation) and later "Substance P" which it still is called. A few years afterwards I found that it could be salted out and behaved like a polypeptide. Later, B. Pernow in our laboratory found a simple method of obtaining the substance in a high degree of purification.

Thus almost the first turn of the spade brought up a new biologically active factor which certainly was to a large part due to luck. Whatever the cause, it had a strongly encouraging effect on the young scientist. Clearly, the demonstration of a new compound with certain actions only constituted the introductory step, and it remained to show what possible physiological function it could have. Definite conclusions as regards the physiological role of Substance P have still not been reached, but its occurrence in the gut, its high biological activity, and its atropine resistance would make it a strong candidate for a motility hormone of the gut, rather than acetylcholine.

These early experiments not only whetted the appetite for finding more active substances in biological material but also provided the necessary "know-how" for making such attempts successful. Competition was strong, however, and this was the time for new unidentified biologically active substances to appear in large numbers. Some of these later proved to be mixtures of known substances or the result of misinterpreted effects.

On my return to the Pharmacological Department of the Karolinska Institute in the early thirties, further studies of the biological action of tissue extracts led to the observation of what appeared to be adrenaline in the prostate gland. J. B. Collip had made similar observations and carried the purification of the active sympathomimetic factor further. He arrived finally at the conclusion that it might be tyramine. Considering that this amine releases noradrenaline, which is indeed the active factor in the vesicular gland, the conclusion was not far from the truth.

There was then only a short step to testing seminal fluid in 1934. The lowering effect of a small volume of the native material on the urethane-treated rabbit's blood pressure was truly startling, and again suggested a new active principle. Learning that M. W. Goldblatt in England had published a note of some similar results in a little known Journal the year before was encouraging and provided at the same time a challenge to continue these studies. A systematic study of prostate and vesicular glands from various animals gave the surprising result that, except for human material, only the sheep vesicular gland contained the new factor in large amounts.

In the course of the purification work it became clear that the active factor was of lipidic character, and it could soon be characterized as an unsaturated lipid soluble acid which was named prostaglandin. This was definitely proven with Professor Hugo Theorell's ingenious electrophoresis apparatus, which made it possible to follow the mobility of the active principle. The oily material fortunately yielded a watersoluble barium salt on addition of barium hydroxide which precipitated large amounts of impurities, and on desiccation it gave a dry amorphous powder, suitable for storage. From a large batch of vesicular glands from sheep collected by the helpful Icelandic Slaughter Company enough material could be obtained to serve as starting material for further purification work and biological tests. It was also natural to approach the lipid specialist S. Bergström, who soon became interested in the purification problem. After long and systematic work he not only succeeded in isolating several members of the prostaglandin family but also tackled the intricate structural problem, a masterpiece of chemical knowledge and skill.

I believe these events illustrate in a convincing way both the value of early training in an outstanding laboratory like H. H. Dale's, and the great advantage of belonging to a school fostering scientists like Theorell and Bergström. Under less fortunate circumstances the basic observations might not have been made, and the subsequent development not achieved in our research groups.

At the time of its discovery in 1934 and in the next few years afterwards the prostaglandin was mainly regarded as a curiosity, and in the interval passing until its isolation by Bergström and his group in 1960, other interests came into the foreground. The excretion of amines in the urine either as free amines or as conjugates had been demonstrated by several laboratories and it seemed tempting to look further into this field. Before long the biological tests revealed a nicotine-like substance in urine. I rejected the suggestion of my friend Irvine Page that is was just nicotine, since it occurred not only in the urine of my two boys, then 8 and 10 years of age, but also in bovine urine. This stimulated my interest and the following year, 1944, the active compound was isolated and identified—it was piperidine. This might have started off a systematic study of its occurrence in the body and its formation, but some other results claimed preferential interest since they appeared to be of special significance.

The story of the different "sympathins," told by Cannon and Rosenblueth in the early thirties raised great interest as a continuation of the discovery of chemical transmitters by Loewi and Cannon. The situation became increasingly more intriguing, however, by some observations made by other research workers. Bacq's hypothesis in 1933 that "Sympathin E" might be identical with noradrenaline was not accepted by Cannon and Rosenblueth, however. As late as 1939 Cannon concluded that tissue sympathin actually was adrenaline, as generally held at that time.

This was evidently a field offering some chances of obtaining more information by analysis of tissue or nerve extracts. Such extracts in our experiments clearly showed the presence of an adrenaline-like substance, but it became gradually clear that the activity pattern did not wholly agree with that of adrenaline. Could it be noradrenaline? This was a real challenge, sweeping away other seemingly promising research projects. It must also be remembered that at this time research funds were very limited and teaching took a good deal of the available time. It was necessary to select one field and not split the resources on several topics.

Once the suspicion had been raised that the nerve transmitter was different from adrenaline it became easier to design the right kind of experiments. It then turned out that the results fitted in very nicely with the assumption of noradrenaline as the active catecholamine in adrenergically innervated organs.

An attempt to check the possibility of noradrenaline occurring in the adrenal medulla offered an instructive experience. For this purpose I used an extract of rabbit adrenals which were easily available in the laboratory. The result was perfectly clear: the active amine was adrenaline. Not knowing that I had picked the only mammal with practically only adrenaline in its suprarenals, I had a good chance to consider the dangers of generalizations in the following year, when Peter Holtz' work on noradrenaline in cat adrenals was published.

The hypothesis of noradrenaline as adrenergic neurotransmitter did not in the beginning meet with great credence, probably because of the authority of those who regarded adrenaline as transmitter. This was perhaps to be expected and saved me from what might have been a disappointment. A latency period of a couple of years before the findings and the implications would be generally accepted would seem almost unavoidable. As usual some scientists at once saw the significance, like B. A. Houssay, while others only reluctantly were prepared to accept the fairly obvious evidence on which the concept was based or continued to use the term "Sympathin." On the other hand it is, I think, a common observation that some new theories, results, and concepts which are, at least to some people, clearly doubtful or erroneous, may readily find their way into textbooks. However, interest grew rapidly, particularly in the U.S.A. and in England, where my friend Gordon Wolstenholme arranged several Ciba Symposia on various branches of the new area. The differential analysis of adrenaline and noradrenaline in urine, and the development of useful biological and chemical methods of assay no doubt stimulated the growth of this field.

If the neurotransmitter was present in the adrenergic axons, as we knew it was, how could it survive there in constant amounts? This was a question that had to be answered. The electron microscopic findings of two pioneers in adrenergic nerve biochemistry (H. Blaschko) and morphology (N. Å. Hillarp) seemed to provide the answer. Why should not subcellular particles rich in noradrenaline occur in adrenergic nerve axons if they could occur in the homologous chromaffin cells? Hillarp at once responded to my proposal of looking for "granules" in adrenergic nerves that were rich in noradrenaline, and before long we had the necessary data to express the view that the adrenergic neurotransmitter was stored in a protected form in the axons, and consequently in all organs supplied with adrenergic nerves.

The isolated nerve or organ granules have subsequently served as a readily available material for studies of their properties in several laboratories including our own, and their multi-faceted reaction pattern to various drugs has gradually helped to elucidate several types of drug-action on adrenergic activity. The enormous growth of the number of studies in this field, regarding both the CNS and the periphery, makes it increasingly difficult to maintain a clear view of the field. Still, the mechanism behind the release of the adrenergic neurotransmitter is a challenging problem and we stand here before events that take place at a level close to molecular biology. Although knowledge about autonomic neurotransmission has advanced conspicuously in a brief period of some 25 years I feel personally that some day, perhaps in the next 10 years, an increased insight into molecular and membrane biology will allow us to see more clearly how the transmitter is liberated from the axon. A salient point is still: If acetylcholine is implicated, where is its place?

Only rarely the research worker is able to make contributions that are so rounded off that they seem to form a closed chapter. Perhaps this is more true for physiology than for other disciplines. Very often there is a system of cross-connections that seem to extend in all directions. As a finishing illustration I shall only mention one such cross-connection that appears to bind together two fields in which I have been specially interested. I am referring to the release of prostaglandins on adrenergic nerve stimulation and the action of the PGs on the effect of such stimulation. The recent finding in our laboratory (P. Hedqvist) that prostaglandin in minute concentrations can under certain conditions block adrenergic nerve transmission represents, I think, an example of an unexpected crosslink between two systems. Thus research impulses leading to new and useful combinations clearly depend on the environment. Variations in the "programming" of the scientists would seem to be a prerequisite for ensuring a healthy variability of the research.

Sometimes even apparently lackluster and uninspired, tedious work may give good information. Certainly it appeared a dull preoccupation to determine the noradrenaline content of a large number of organs and tissues in the body, but it did reveal some facts of importance such as the presence of the neurotransmitter in the brain, and the occurrence of remarkably high amounts in the male accessory glands. Finally it offered a means of determining the relative supply of adrenergic nerve fibers to different organs, as confirmed by the later developed histochemical fluorescence technique.

Even if testing extracts of various tissues and organs on a battery of pharmacological test objects may not be characterized as sophisticated science, it can lead to valuable findings, often not predictable. It is perhaps worth recalling that research in Portuguese is called "pezquisas," which literally means fishing. For those who have a feeling for where to fish and recognize a good fish when they see it, this method has its merits. On the other hand the intellectual pleasure behind an intelligently planned experiment giving the desired answer is great and well earned.

Which advice, if any, should be given to young scientists as regards research procedures in a wider meaning? Considering the large number of people actively engaged in research at the present time one can be reasonably sure of two things. One is that a large proportion of these will develop into good and useful conventional scientists, using available techniques on generally accepted problems, and applying good statistics to the required number of experiments. The results of these efforts occupy a large part of the space in the growing number of journals. Much of this work is of confirmatory type and would be useful but for the often too large number of pages. A tendency in papers of this type to expand on long and tedious discussions is noticeable.

For a young scientist it is of great concern that he should be recognized by those who decide the distribution of grants and handle the applications for positions. A board of first rate scientists generally chooses the best of the young generation for grants and positions, thereby tending to perpetuate itself, which, alas, may be said also of a board of less distinguished members. In a large country with many universities and research institutions the situation is never serious, but in a small country the swing in either direction may be very marked. In fact a few authoritative persons of the convincing type may on questionable grounds promote certain types of research and hold back others. The young scientist therefore may have to adjust his work, or at least his program, so that it fits in the prevailing pattern. It is thus tempting for a young research worker to fall in line with a reasonably profitable line of research which has reached a state of general acknowledgement. It is safe; and since work is going on in the field there are good chances to be quoted, often in connection with a recognized colleague. The population of research workers has sometimes been compared to a heap of soapbubbles. When a small one collides with a big one it just unifies with the large one and makes this increase.

The advice given to young scientists, approaching the entrance of the temple of science, had formerly often an idealistic touch, and to a previous generation, more accustomed to sermons, the wise men were expected to preach on chosen occasions. No doubt the present generation of young scientists is less amenable to listen to the big words; they look at their job in a more practical way and their primary thoughts are often more concerned with salary than research philosophy. They know that in order to proceed along the career road they must have publications in sufficient number. Consequently they are tempted to prefer investigations within an established field using techniques that are acknowledged, and concepts that are accepted by the Granting Boards. Such studies will often lead to the desired position without too original thoughts or lucky findings. Unfortunately the products will often bear a slight mark of safe banality stamped upon them although they serve their purpose in other respects. The harsh competition in fact appears to foster a new kind of research worker who is more like an engineer who produces numerous data with the aid of sophisticated machines.

A recurring dilemma for any scientist is to know when to go on with a problem and when to leave it. This also implies that those who are responsible for directing or financing other people's work must judge the probability of success or failure. When a scientist builds up a hypothesis on the basis of his own data and those of others, in pharmacology as in other biological sciences, the process mostly rests on the more or less solid ground of analogies. To decide whether a new hypothesis is likely or not is a formidable task that requires judicious weighing of a large number of factors, which can hardly be assessed on a precise basis. There is little doubt, however, that some prominent scientists have a great ability to separate the gold nuggets from the uninteresting material and have a "feeling" for the probability of a hypothesis or theory, and for its significance.

Obviously some scientists have the gift of selecting topics and problems better than others. When working in Sir Henry Dale's laboratory in London in the thirties we used to say that "a guess by Sir Henry is more likely to be right than many so-called established facts from other sources." In other words, we had accepted the use of probability without evidence. This way of proceeding has dangers but as long as there are limits to man hours and funds this method has its virtues and experience seems to verify this. Of the numerous suggestions and theories put forward only those will be selected for closer study which appear to be likely. It is of course inevitable that this occasionally leads to overlooking single projects that should be followed up, but on the whole this procedure has many advantages.

The fast growth of the volume of scientific achievements has been shown to follow an exponential function, doubling every 10 to 15 years. This applies to the number of individuals engaged in the field, as well as to the number of journals and other parameters, and varies only to a small degree between different subjects and different countries. The rapid growth of pharmacology, like other sciences, has some interesting corollaries. Not only does the exponential increase mean that about one-half of all scientists who have ever existed on our planet have worked for less than a dozen years, but—on a quantitative basis—one-half of our accumulated total knowledge or, let us say the number of data, is the result of research work done in the last decade. No wonder it is hard to keep track of them. Considering this menacing avalanche of data it becomes a "must" to present them in as "pure" form as possible, freed from all unnecessary outgrowths. Some journals adhere successfully to this principle for the reader's benefit, but much remains to be done. Overdocumentation and verbosity are still too frequently encountered. For the young scientist it is often a disappointing fact that he is rarely quoted unless by himself, and his papers are mostly rapidly buried in an ever increasing stream of publications. On the other hand it is increasingly evident that a small number of papers are quoted over and over again.

To observe the events and trends in a research field is sometimes fascinating, often interesting and even intriguing. At times one is reminded of a flock of starlings rapidly switching from one direction to another, following some directional signals unseen to the watcher. Sometimes an idea is "fashionable" and will rapidly ensure the adherence of a large group. It can be the correct one for that matter, but the opposite may happen. To turn the tide in such cases is often a slow and tiresome process. Controversial opinions—even with the wrong one at the top—are not necessarily an unwanted state of affairs since they may challenge the ingenuity and experimental skill of the proponents and lead to a quicker solution of the problem. Tenacity in maintaining fixed positions and unwillingness to accept evidence from the counterpart are often characteristic features in this game, however.

The diversity of problems and difficulty of following the work done in other sectors tend to limit the outlook for the experimentalist. Many attempts have therefore been made to integrate the findings in different or at least adjacent fields to a more complete picture. To take an example: in pharmacology as in physiology one of the most pertinent tasks is to solve the problems of interaction between agonists, antagonists, and receptors. Protein and lipoprotein chemistry, combined with morphological studies on membranes and electrophysiological experiments revealing ion currents are here likely to give useful information. The increasing necessity to consider events at a subcellular or molecular level is apt to bring new and perhaps unforeseen difficulties of conception. We have still a very incomplete idea how the molecules move and shift in their microworld; the consistency and geography of the cytoplasm is still imperfectly known and so are the molecular pathways within the cell. The time scale is another complicating factor. We know that on depolarization of the axon membrane at the neuro-muscular junction a momentary shift in calcium ion distribution occurs, followed by a release of the transmitter acetylcholine, which on the other side of the synaptic "cleft" reacts with receptors on the muscle in the endplate region of the muscle cell. All this occurs within a millisecond, as shown by the beautiful studies of Katz and others. Increased knowledge of the kinetics of fast reactions in organic media are here a prerequisite for better understanding of the events.

Of the many new research fields associated with recent developments in the neurotransmission area perhaps the central actions, including psychopharmacology, have the most far-reaching consequences. This was predicted by H. H. Dale who wrote (about acetylcholine) in his Nobel Lecture in 1936: "The possible importance of such an extension, even for practical medicine and therapeutics, could hardly be overestimated." The discovery by B. B. Brodie that after administration of reserpine amines were no longer stored in the brain in the normal way and that these disturbances were associated with psychic alterations was a breakthrough in this field. We are witnessing a steady progress in psychopharmacology, and one might predict that by the judicial use of drugs it will be possible to adjust the mental instrument to considerable extent. There is no need to emphasize what can be achieved by improper use of such tools.

The steadily increasing number of scientists in the biological as in other fields might perhaps suggest that they should be increasingly powerful as a body, particularly considering the importance of the biological sciences for human welfare. Recognizing their own potential value, biologists sometimes express the view that they should be called upon to a greater extent to take an active part in the handling of the city's, the nation's, or the world's affairs.

It would not be difficult to find a large number of highly competent, honest, and hardworking scientists, willing to assume such a role as advisers to the population in toto, and yet the number serving in such capacities is very limited to say the least. The reason is of course that those in power—regardless of the nomenclature of the system—are for the most part only moderately interested in aspects of science other than those that can serve their political aims. In such cases a suitable expert can always be found, serving both as a proof of recognition of research as such and as a scientific support for a specific purpose. It may be well to recognize that this situation is not likely to change and that the ambitions of the scientist should profitably be linked also in other directions.

Although research work is a job like many other kinds of work it is often so engaging that it may indeed sucessfully compete with most hobbies. My revered master and friend Bernardo Houssay, on the third day of his holidays at the seaside wrote a postcard to the laboratory: "I envy you who can stay in the lab and do research." This love for the work makes it possible to find individuals who are willing to do high quality work at a modest remuneration.

Research requires many co-factors in addition to hard work and some luck in order to be fruitful. Perhaps one might use a travesty: "En recherchant, il faut être dégagé de toute autre préoccupation." Everyone engaged in research recognizes the importance of a good research climate. To define this is not easy but it does include a certain amount of freedom of time and freedom from want, a reasonable supply of equipment and means to continue along unexpected lines, relatively loose ties as regards research program and so forth. These requirements may seem too liberal for many rationalists and authorized research planners and for those who allocate the money, yet there is no guarantee that even under these favorable conditions anything really worth while will turn up for a long time. Fortunately, there are almost always some products of work that can be accepted as a reasonable return for the money spent.

Looking back, it is fairly obvious that the past 50 years have been a favorable time for research, reasonably unhampered by such interference as might check the freedom and choice of subjects. It is not equally clear that this will be the case in the future. An increasing tendency to control the activities can be noticed and this may be felt as a tether that does not encourage those to enter the field for which a certain freedom is a prerequisite for successful work. It is to be hoped that even in the future a certain liberty will be allowed in this respect.

Most of the reflections laid down in this article have been said before and probably better, but the experience of those who have been in research for a long time and who have met scientists of all kinds and calibers may still have an interest for others who are in the beginning of a research career. After all, homeostatic mechanisms operate also in the human mind, and certain basic rules are likely to remain unaltered, even if a variety of external factors may produce temporary changes in the outlook.