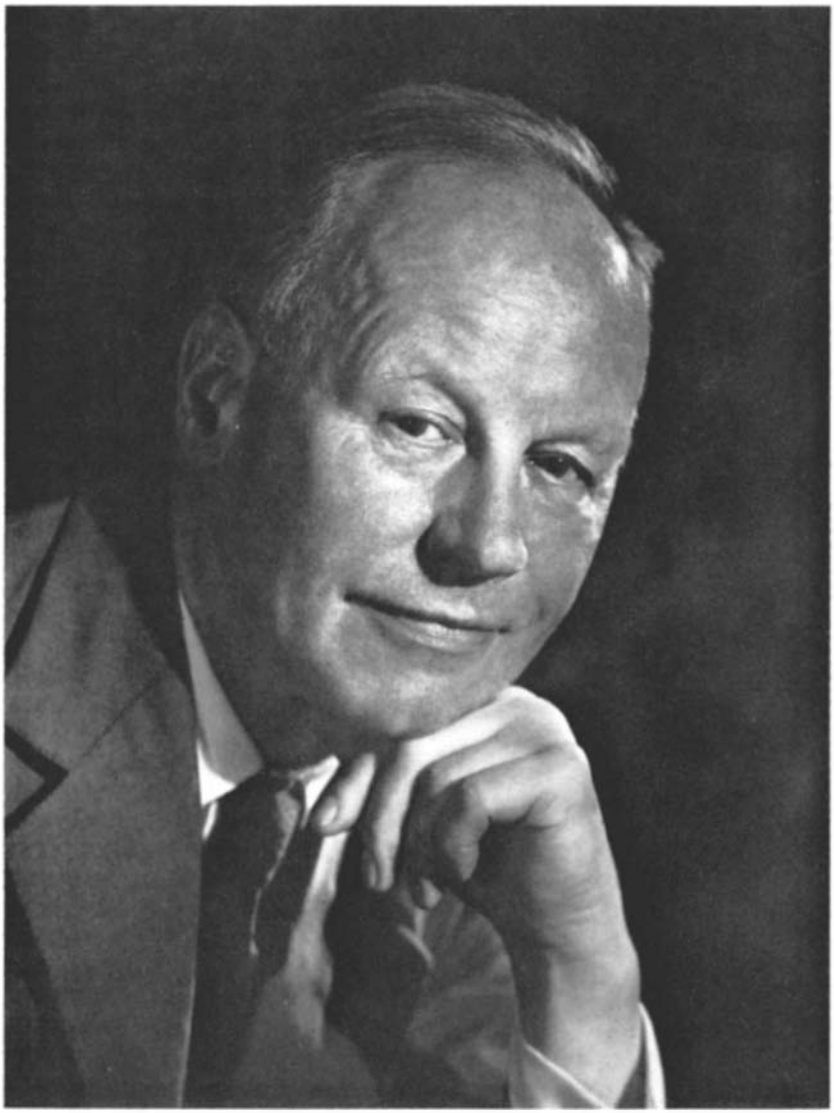

J. Harold Burn

ESSENTIAL PHARMACOLOGY

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The beginning.—When I went to Cambridge in 1909, I became a member of Emmanuel College, of which John Harvard had been a member some 300 years earlier. I had the good fortune to have as a tutor, whose duty it was to supervise my progress in a general way, a man called F. G. Hopkins! He was the pioneer biochemist who isolated tryptophan, who worked on what he called "accessory food factors," and discovered glutathione. He was one of the most modest, gracious and graceful men I have ever known.

At Cambridge I expected to make Chemistry my principal subject, but I found that for the first two years, I had to read Physics and two other subjects as well. With Hopkins' approval Physiology and Botany were chosen. I discovered that, compared with those who taught Physiology and Physics, the chemists were an unattractive lot, particularly those who demonstrated in the practical class. These seemed very undistinguished, had cheerless faces and wore rather shabby clothes. The physiologists and the physicists had far more personality. In Physics I heard lectures given by J. J. Thomson, who seemed to be not of this world, and one of the demonstrators was Aston who built the first mass spectrograph. In Physiology the Professor was Langley, always well-dressed, who rode to hounds (that is he hunted the fox) and spoke to no one so far as I could see. There was W. B. Hardy who, when not studying the behaviour of colloids, sailed a yacht in the rough seas of the English Channel. In the summer he pinned a notice on his door which said "Back tomorrow." Joseph Barcroft, an Ulsterman, also sailed boats, and Keith Lucas, who was in part an engineer, had a large motor vessel on the Cam. Hidden in his room was a young man called Adrian, who spent his spare time at one period painting pictures in the style of Marinetti for an exhibition. It is said that all the works were sold, mostly to eager, but unwary Faculty members.

Then there was the striking athletic figure of A. V. Hill, the very distinguished-looking W. M. Fletcher, and finally, helping Barcroft and Hopkins to fill the place with humanity, was H. K. Anderson.

To enter the Cambridge world after leaving the school world was like meeting sea breezes on the shore after being confined in a stuffy room, and for two years I was mainly interested in debates and discussions of politics, religion and literature, which at least taught me to form my own opinions. The result in the examinations at the end of the second year was, not surprisingly, a disaster, and this made it difficult to decide what subject I

should choose for my final year. The choice would determine my future life. I discussed it all with Hopkins, saying that Physiology was the subject I preferred. He thought that if I wanted to have some immediate security after my third year, I had better choose Chemistry, since there was always a market for chemists. But if I was prepared to leave the future to take care of itself, then I might choose Physiology, despite the fact that I was not a medical student. He advised me that I must not read text-books, but should study original papers, and should read them critically. He mentioned that I should look at the papers of H. H. Dale and P. P. Laidlaw. When I did so, I felt that they were of little use for the purpose of my final examination, because I could find nothing to criticize! At that time I was unable to see what a break into a new world they represented.

After my third year I began research with Barcroft on two small problems concerned with the oxygen capacity of haemoglobin. I learnt (to my surprise) that I could work accurately, but I was still only half-interested and was occupied with other things. I was secretary of a University Social Discussion Society, and of its Inner Circle which invited distinguished public figures to address them. One of these figures I remember was Beatrice Webb who had encyclopaedic knowledge on the reform of the Poor Law. I may say that she stayed, unforgettably, at the house of Mr. W. C. Dampier Whetham, and that he lived at Upwater Lodge, where I had dinner.

Barcroft, irritated no doubt by my wandering inclinations, told me one day in his ever-genial manner that what I needed was three years of a really hard time. When I was in the Army during the First War, I got them. Barcroft was easily the best lecturer in the department, and gave fascinating demonstrations (assisted by John). One, of which I have a photograph, was made on an anaesthetized cat in which a cannula had been inserted in Wharton's duct to collect the saliva. After an inch or two the cannula turned vertically upwards, and when the chorda tympani nerve was stimulated the saliva rose up the tube. There was also a cannula in the carotid artery, which turned vertically upwards as well, so that the blood (prevented from clotting by hirudin) ran high up this tube and oscillated between its systolic and diastolic height at a mean level of about 130 cm. The question which Barcroft then asked the students was whether continued stimulation of the chorda would drive the saliva above the height of the blood pressure. How many know the answer to that question?

So successful were Barcroft's demonstrations that when I went to Oxford in 1937, Edith Bülbring and I began a series of mammalian demonstrations there, and I believe they continue. I, for my part, still carry out about eight demonstrations each year in the Department of Pharmacology, Washington University, St. Louis, and there is a proposal that next year each group of four students be given a demonstration to practise until they can do it successfully, and then demonstrate it to the others, using closed-circuit television.

The main trouble in devising experiments (which are not biochemical) for a practical course in Pharmacology is that the student has to discover how to assemble his equipment and set up his preparation before making his observations. Too many fail to complete their work in time, and as a result feel frustrated. But if a small group remain with one experiment until they have mastered it, they learn much more from it, and can demonstrate it to others. They can even make a heart-lung preparation in the dog, and gain great satisfaction from doing so. The experiments must, however, be chosen with care.

Plunge into Pharmacology.—It was on January 1st 1914 that I went to work with H. H. Dale. To leave the strictly monastic life of Cambridge for the gayer life of London was exciting. The atmosphere did not conduce to single-minded devotion to the laboratory, and I can remember often feeling worn-out at the end of a morning's experiment, when Dale was obviously as fresh as at 10 a.m. But I was very happy to have arrived where mammalian experiments were done, and where, moreover, they were done in great style. Much has been said about the importance of Dale's discoveries, but not many have commented on their presentation. His paper "On some physiological actions of ergot" (1) is a good example, and Figure 26 is one that could be enlarged and chosen to adorn the drawing-room wall of an obstetrician as being a work of art. (It is the original demonstration of the action of pituitary extract on the uterus). All the kymograph tracings right up to 1936 were marked by their elegance, and few workers have been able to use the plethysmograph with such success, and to write with white ink on the varnished paper so beautifully as Dale.

All members of the staff of the Wellcome Physiological Research Laboratories had lunch together, and I soon realized that daily lunch is an essential part of laboratory life if workers are to profit by what they can learn from each other.

My work introduced me to the spinal cat and to the isolated organ bath, and these remained standard preparations for many investigations. Some workers forget that responses to autonomic nerve stimulation are greatly reduced in animals anaesthetized with pentobarbital.

Dale left the laboratories at the end of June 1914, and in August the First War began. I had been in Germany in 1913 and in Austria in 1914, with the result that during the first three months after war was declared I was very much anti-war, and had furious discussions, particularly with G. S. Walpole at the lunch table. The sudden development of a fierce hatred for Germans seemed entirely artificial. However, by October I began to feel that if everyone disagreed with me, I might be the one who was wrong, and that if I wanted to see a battlefield, I'd better get into it before it was all over. By good fortune a Cambridge Don invited me to join his Signal Company, and in July 1915 I found myself in France in charge of the Section of the 12th Division Signal Company which was attached to the 36th Infantry Brigade. The duty of the Section was to maintain telephone commu-

nication between the Brigadier-General and his four Battalion commanders. This meant plenty of shell-fire, but not taking part in an attack.

I then discovered all about courage, and how surprisingly right Bernard Shaw was in his play "Arms and the Man." In it an officer fighting for the Serbians against the Bulgarians runs away, and finds himself taking refuge in a lady's bedroom. She refuses to be frightened by him, and says "though I am only a woman, I think I am at heart as brave as you." He replies to her "I should think so. You haven't been under fire for three days as I have. I can stand two days without showing it much; but no man can stand three days: I'm as nervous as a mouse."

After being away from the trenches and the war for a few weeks, you could be quite indifferent to the bursts of shells and to machine gun bullets for 24 hours. But after that these things again had their effect in restricting your movements. The men who got the medals were those who could go fearlessly into action, capture a trench or destroy a machine gun, get wounded and then be out of the line for three months. Then they could return and repeat their brave deeds and again get wounded. The important thing, however, was not to be killed.

Before joining the army in October 1914, Dale suggested that I would be wise to take a medical degree, and I registered as a medical student who had done part of the course. In December 1917 I was able to leave the Army to do my Anatomy and clinical work at Guy's Hospital. Because the staff was depleted by the war, the opportunity for students was great. While I was a surgical dresser, I was told one afternoon that I must begin at once as an extern and visit patients in labour in their homes in the district around the Hospital. I was given a small bag and told to go to 2 Farthing Alley, Dockhead. I protested that I had had no instruction in Obstetrics, but I was told I must do my best.

At that time (December 1919) there were almost no qualified midwives, and when I arrived at the house, the patient was certainly in labour, attended by a Mrs. Gamp, straight from the pages of Charles Dickens. Before long a baby was born, and after cutting the umbilical cord, I put on a dressing. Then to my bewilderment, the mother delivered an object rather like a football. The only conclusion I could come to was that this was some kind of placenta, and I placed it in the chamber pot. About ten minutes later the mother delivered what was definitely a placenta, and I wondered what I had put in the pot. I took it out again and realized that it was a second baby born inside a caul. I removed the baby from the caul and all was well. Three days later both mother and twins were progressing satisfactorily. I did duty as an extern for five weeks, during which I attended 68 deliveries, including seven breech presentations, and one face presentation among other anomalies.

In September 1920 I rejoined Dale at the National Institute for Medical Research, where he was the head of the Department of Pharmacology and Biochemistry. My special duty was to study methods of biological standardi-

zation. The lunch-time conversations with the bacteriologists and pathologists were often interesting and informative. The bacteriologists were very much under the influence of Almroth Wright and had no thought of making a chemical approach to the killing of bacteria in the body. In 1911 the ethyl derivative of hydrocupreine had been found to be potent in killing them outside the body, and indeed when given to mice it protected them against a general infection with pneumococci. But in doing so it caused blindness. I remember Clifford Dobell arguing that any substance which killed bacteria would kill man. But probably the real difficulty was that the bacteriologists had no knowledge of Chemistry.

In addition to my special duty of working on biological standardization, in which subject I showed no sign of having ideas of my own, I learnt much from Dale by working with him both on the action of insulin and on the action of histamine. All these things helped me to acquire more knowledge of methods. On January 1st 1926 I went to take charge of a new laboratory at the Pharmaceutical Society in London which was set up mainly to study methods of biological standardization. There was an interesting development which concerned the alkaloids present in ergot. Very few today know the sequence of events which led to the discovery of ergometrine (also called ergonovine and ergobasine).

The ergot alkaloids.—In 1922 Dale (2) opened a discussion at the Royal Society of Medicine concerning Liquid Extract of Ergot, and the active substances in it. He recalled that Tanret in 1875 obtained a pure crystalline alkaloid from ergot which he called ergotinine; this was associated with an amorphous substance having the same chemical composition; it had a low specific rotation. The amorphous substance was thought to be ergotinine also. The crystalline alkaloid was naturally chosen for investigation, and since it was found to be inactive pharmacologically, the inference was drawn that it was also inactive therapeutically. However Barger and Carr found that the amorphous substance was not in fact similar in composition to the crystalline ergotinine and that it was an intensely active substance. They called it ergotoxine. Dale said that "from tracings of experiments conducted in the laboratory, ergotoxine might have been regarded as a stimulant of plain muscle responsible for the therapeutic action of ergot." The method described in the British Pharmacopoeia of 1914 involved the extraction of ergot with water instead of alcohol, which was used in the previous Pharmacopoeia. Since ergotoxine was not soluble in water, it followed that the method "might almost have been designed to exclude ergotoxine." There were indeed other active substances in the watery extract—tyramine and histamine. But tyramine could be cheaply prepared from tyrosine and histamine from cheese. They were not substances peculiar to ergot. The charge brought against those responsible for the British Pharmacopoeia 1914, was that they did not understand what they were doing. When watery extracts were prepared, the specific active substances were thrown away and the adventitious substances were retained.

The Secretary of the Pharmacopoeia Committee, Tirard, was present at this discussion, and said that 19 licensing authorities had been consulted, and not one of them had suggested omitting any preparation of ergot. A. W. Bourne, obstetrician at Queen Charlotte's Hospital, suggested that to resolve this question there should be clinical trials, using methods enabling measurements to be made, and the results might then replace clinical impressions.

Sometime in 1923 or 1924 I was asked by Dale to assist Bourne in carrying out some work of this kind, but for one reason or another the collaboration was brief, though long enough to show that a method for recording the intrauterine pressure in women in labour was practicable.

The Geneva Conference of 1925.—In 1925 the League of Nations Health Organization held a Conference in Geneva, at which Dale presided, to obtain international agreement on stable standards for insulin and other therapeutic substances "the purity and potency of which could not be determined by chemical means." Extract of the posterior lobe of the pituitary gland was one of these. For each substance a unit was chosen in terms of the activity present in a given weight of the powder which was to be the international standard. The standard powder for pituitary (posterior lobe) extract was declared by members of the Conference to contain 1 unit of activity in 0.5 mg.

When I went to the Pharmaceutical Society in 1926, it seemed to me that it would be fitting to attempt to determine what clinical effect would be produced by 1 unit. With Dale's agreement I approached Aleck Bourne again, and we proceeded further with observations on women in labour. A small sterile rubber bag attached to a sterile catheter was passed into the uterus when the os was sufficiently dilated. The bag and catheter were filled with water and connected by a long rubber tube to a mercury manometer, so that a rise of pressure within the uterus could be recorded on a drum. We discovered that in some patients the subcutaneous injection of two units produced as large and prolonged a contraction as was ever desirable, and were therefore able to suggest that this should be regarded as the maximum single dose. However, in some patients this dose had a very small effect. Our investigations therefore had little useful result, for it did not occur to us to try giving the extract by slow intravenous infusion. The intravenous drip was not invented until 1935, though we might have invented it ourselves.

Having made our observations on pituitary extract, we then decided to turn our attention to ergot, and to determine which was the important constituent. The task seemed simple. All we had to do was to examine the three substances, ergotoxine, tyramine, and histamine. This seemed preferable to spending time testing the effect of an extract itself. An extract would have to be given by mouth, and it would be difficult to know when its effect began because of the time required for absorption from the alimentary tract. The active constituents, however, could be given by injection, and any effect they had should be seen without delay. The only difficulty was about ergo-

toxine, which was not on sale. This was solved by using ergotamine instead, which had been isolated by Stoll in 1921 from ergot growing on Festuca grass. A careful comparison of ergotamine with ergotoxine had been carried out by Dale and Spiro. The result was that the two substances were indistinguishable by any test which could be applied. I myself made several comparisons by the method of Broom and Clark, using the reversal of the action of adrenaline on strips of the rabbit uterus. I found no certain difference between the two substances in any comparison.

Investigation showed that tyramine was without effect on the uterus of a patient in labour, and histamine, in the amount present in the full dose of an ergot extract, was also without effect. Ergotamine, however, when injected in a dose of 1 mg., produced a powerful contraction of the uterus lasting at least 16 hours. The results were taken to mean that a Liquid Extract of Ergot must be prepared so as to contain ergotamine or ergotoxine.

There was, however, one dissatisfied person. This was F. H. Carr, who together with G. Barger isolated ergotoxine. He was very anxious to know whether the effect of ergotoxine was actually the same in a patient as the effect of ergotamine. We did not feel able to make this comparison, because we had been warned by our experience with ergotamine that such trials on patients in labour were not without risk to the foetus.

However, Chassar Moir undertook the task under different conditions. He worked on patients in the puerperium after all the stages of labour were complete, and the foetus and placenta were delivered. He was then able to show that ergotamine and ergotoxine were indistinguishable, and thus he satisfied Carr. What was Chassar Moir to do then? It seemed as if he was left with nothing to do but dot the i's and cross the t's. He decided to have a watery extract prepared according to the 1914 British Pharmacopoeia, which would contain no ergotoxine or ergotamine, and then he gave it by mouth to one of his patients. I quote from his account (3).

> "Judged by all previous work, this preparation ought to have been inert, for analysis showed that it contained only a trace of alkaloid. The extract was used in 3 cases in doses of 4, 3, and 2 drachms. It was with the greatest surprise I found that far from being inert, this preparation surpassed by great measure the activity of any drug which I had previously used in the same manner. An equally surprising fact was that the effect appeared in a remarkably short time. In one case only 4 minutes elapsed between the swallowing of the extract and the onset of powerful uterine contractions."

Chassar Moir had discovered the presence of a new active principle in ergot which was soon realized to be the substance responsible for the usefulness of ergot in midwifery. That was in 1932. Chassar Moir and H. W. Dudley isolated the alkaloid in 1935, and gave it the name of ergometrine. Others were excited by Chassar Moir's discovery, and the alkaloid was also isolated in 1935 by Stoll who gave it the name of ergobasine, and by Dragstedt who gave it the name of ergonovine.

The story was a splendid cautionary tale. It warned us (*a*) that the impressions of clinicians should be treated with respect; (*b*) that because one specific active principle has been found, it must not be assumed that there is not another; and (*c*) that control observations, however unnecessary they may seem, should never be omitted. If we had once given a dose of the 1914 B.P. Liquid Extract of Ergot to a patient by mouth, we would have discovered the new alkaloid ourselves.

The use of a perfusion pump.—Having given an account of a research which was a failure, and from which I could claim no credit whatever, I feel free to turn to a different investigation of which the outcome was more satisfactory. During the last year I worked with Dale a further study was made of the vasodilator action of histamine, following his classical paper published jointly with A. N. Richards in 1919. This work was done in the main by using a perfusion pump with the object of reproducing in the perfused leg of the cat effects of histamine which were regularly observed in the whole animal. The experiments were sometimes successful and sometimes not, and obviously there were factors in a perfusion experiment which were only discovered by perseverance.

When I went to the Pharmaceutical Society in 1926, I intended to do further work of this kind, and when Dale and Schuster produced a new pump in 1928, I obtained one and proceeded to investigate the action of tyramine. There was one reason for choosing tyramine, namely that Tainter & Chang (4) had shown that it differed from adrenaline in its relation to cocaine. Whereas Fröhlich & Loewi (5) observed that the rise of blood pressure produced by adrenaline was increased in height and duration in the presence of cocaine, Tainter found that the rise of pressure produced by tyramine was diminished or even abolished by cocaine. The difference suggested that adrenaline and tyramine did not produce a rise of blood pressure in the same way.

The Dale-Schuster apparatus was a double pump designed to deliver blood to the organ to be perfused, after which the blood was collected from the venous outflow and pumped through the lungs for re-oxygenation. The procedure was to prepare one hindleg of a dog for perfusion through the external iliac artery, then to bleed out the dog, and then to prepare the lungs. This meant that the leg to be perfused was without a circulation of blood for about 40 minutes, until the lungs were ready and the perfusion could begin.

The results obtained when adrenaline was injected into the cannula tied in the external iliac artery were exactly as expected. There was always vasoconstriction. But when tyramine was injected there was little or no response. Whereas in the spinal cat adrenaline was not more than 40 times stronger than tyramine in causing a given rise of blood pressure, in the perfused hindleg adrenaline was 1200 to 1800 times stronger than tyramine, and sometimes a larger dose of tyramine had a dilator action (6). Why was tyramine so weak in the perfused leg?

Experiments were then carried out to determine the relative constrictor effect of the two substances in spinal cats by observing the volume change produced in a limb enclosed in a plethysmograph; these cats were eviscerated, and the adrenals and kidneys were removed. A wide variation in the relative constrictor effect of the two substances was observed. In some cats tyramine had no constrictor action in the limb at all although an equipressor dose of adrenaline had a good one, while in other cats tyramine caused constriction equivalent to that caused by adrenaline in an amount only 40 times less. Thus the mystery still remained. There was an unknown factor responsible for tyramine having a very variable constrictor action in peripheral vessels.

A fresh attempt to identify this factor was made by setting up a hindleg perfusion in a dog. Beside it a heart-lung preparation was also set up, and when it was working well, the blood from the heart-lung was used to perfuse the hindleg. This was, so to speak, an attempt to synthesize a dog. During the early period in which the hindleg was connected to the heart-lung, there were repeated crises when, because of the persistent vasodilatation in the hindleg, the output of the heart-lung fell. These crises were overcome by injections of adrenaline. As the experiment continued these crises became less serious, and it was then observed that after the immediate effect of an injection of adrenaline had subsided, the response of the hindleg vessels to an injection of tyramine increased. Whereas at the beginning, injections of tyramine into the external iliac artery caused very little vasoconstriction, after the tone in the hindleg vessels had risen and been maintained at a level of about 100 mm Hg. for an hour, injections of tyramine had a much greater constrictor effect than before.

These observations led to the conclusion that the greatest constrictor effect of tyramine seen in the body could also be seen in the perfused hindleg, if there was a steady and continuous infusion of adrenaline into the perfused leg. Thus it appeared that the constrictor effect of tyramine was in some way dependent on the presence of adrenaline. The dependence was not, however, due to the increased tone produced by adrenaline, because the same increase of tone produced by infusing vasopressin did not increase the constrictor effect of tyramine (7).

These results with tyramine led to other experiments in which the effect of stimulation of the sympathetic fibres to the vessels of the perfused hindleg was studied. When a perfusion was started, the stimulation of the sympathetic fibres had little or no effect. When the tone was raised by adding adrenaline drop by drop to the circulating blood until the pressure reached a normal level, stimulation of the sympathetic fibres was then observed to cause vasodilatation. Finally, when the addition of adrenaline was continued for one hour or more, sympathetic stimulation caused vasoconstriction.

The interpretation of the changes was that during the period of preparation in which the hindleg was without a circulation, there was a loss of the transmitter from the sympathetic fibres due to anoxia. Therefore there was

no response to sympathetic stimulation. (Anoxia was later shown to produce a large release of catecholamines from the adrenal medulla.) When the tone was first raised by the addition of adrenaline to the blood, the first change in the response to stimulation was a consequence of the stimulation of hitherto unsuspected vasodilator fibres, which produced a fall in peripheral resistance. When the tone was maintained for one hour or more by the addition of adrenaline, it appeared that adrenaline was taken up into the sympathetic fibres, with the result that when these were stimulated, the adrenaline was released and constriction followed (8).

This interpretation therefore explained the recovery of a normal response to sympathetic stimulation as due to a new phenomenon, namely that of uptake of adrenaline from the blood.

The explanation served to apply to the action of tyramine also, because Tainter and I (9) had found that tyramine, which acted like adrenaline in causing dilatation of the normal pupil of the cat's eye, did not dilate the pupil if the sympathetic fibres had degenerated. In this respect tyramine was completely unlike adrenaline, and the finding that the action of tyramine on the pupil was seen only when the sympathetic fibres were present, suggested that tyramine acted by releasing the transmitter substance from the fibres. The recovery of the constrictor action of tyramine in the perfused hindleg by a long-maintained infusion of adrenaline was thus consistent with the view that the adrenaline was taken up by the sympathetic fibres, and that the constriction produced by tyramine was due to the release of this adrenaline from the fibres. The outcome of this work, done in the early thirties, was unexpected when the work was begun, and the many experiments in which the dog hindleg was perfused eventually provided valuable information.

When, 25 years later, we learned that tyramine has no pressor action in an animal treated with reserpine, we showed in a few days that the pressor action was restored by an infusion of noradrenaline, and we showed also that stimulation of the sympathetic which caused vasodilatation in the perfused hindleg of the reserpine-treated dog, recovered its power to cause vasoconstriction after an infusion of noradrenaline (10). Thereafter the phenomenon of uptake was studied by many.

The training of Ph.D. students.—In the Department of Pharmacology in Oxford, where I went in 1937, I think that most of us might be said to have been under the influence of the discoveries made by Dale and his coworkers in the field of the chemical transmission of nerve impulses. It was these discoveries which enabled pharmacology to contribute so much to medicine as well as to physiology, and there are today many more similar discoveries still to be made. It is surely high time that the transmitter of sensory impulses should be identified, for there is good reason to think that it is liberated into the tissues and probably into the blood stream when stimulation is applied to the cut peripheral end of a sensory nerve (11). The impact of

such a discovery on neurology would certainly be very great. It is possible that the release of the transmitter is a double event, and that the first step is the release of acetylcholine, which then in turn releases the specific agent. But while one would think that many pharmacologists would be trying to discover what this is, in fact it seems that very few of them are doing so, in spite of the problem seeming to be much simpler than the many problems of transmission in the brain.

Our work at Oxford was concerned at an early stage with chemical transmission, and in 1941 Edith Bülbring and I (12) showed that acetylcholine was a transmitter in the spinal cord. We made a preparation of half a dog, having two circuits of blood, one through the spinal cord and a quite separate circuit through the leg. Our observations also showed that the presence of adrenaline in the blood perfusing the cord increased many reflex effects. This action of adrenaline needs more investigation.

To workers who came to the Department to work for a Ph.D., or for a shorter time, we regularly gave problems which involved using isolated organs, preferably innervated. Edith Bülbring (13) devised the isolated rat diaphragm with the phrenic nerve. Dawes (14) prepared isolated rabbit atria so that they could be stimulated to contract at any given rate. He determined the maximum rate of stimulation at which the atria followed the stimuli, and found that this maximum rate was reduced in the presence of quinidine, or of substances with a similar action. The reduction depended on the concentration of quinidine.

Later on, other isolated preparations of the heart were made. McEwen (15) made a preparation of the isolated heart with the vagus nerves, and also one of the isolated atria with the vagus nerves. Huković (16) made a preparation of isolated rabbit atria with sympathetic fibres from the stellate ganglion. Then many learnt to use the preparation of rabbit ear vessels with the postganglionic sympathetic innervation described by Gaddum & Kwiatkowski (17). Those with sufficient skill learnt how to perfuse the superior cervical ganglion by the method of Kibjakow (18). Then there was the Finkleman (19) preparation in which stimulation is applied to the sympathetic fibres running to the small intestine, and finally the still better preparation of the rabbit colon described by Garry & Gillespie (20). In this the sympathetic and parasympathetic supplies can be stimulated separately.

When a chemist has completed his training for the Ph.D. degree, he has a wide experience of the standard chemical procedures. There seems to be much less agreement in pharmacology about what standard pharmacological procedures are. The methods which have been outlined above are among those in which the young pharmacologist can be trained. They are challenging and the skill acquired in learning to use them generally brings a feeling of satisfaction. Pharmacology should be a discipline of its own.

Giving communications.—Whenever anyone in the Department, whether he was a Ph.D. student, or a postdoctoral worker, or a senior member of

the staff, or the Head of the Department, was proposing to give a communication to the British Pharmacological Society, or the Physiological Society, it was the regular custom for him to give his communication beforehand in the Department to as many of his colleagues as wished to come. This was to enable him to rehearse what he was going to say. He must not on any account read his communication, he must speak loud enough to be heard clearly at the back of the lecture theatre and he must say everything in ten minutes or little over. He was recommended to have not more than six slides, and if the slides contained tables of figures, these had to be in large type and to be few. It was common for a person to give two rehearsals, and not rare for him to give three. This often occurred when he did not present his matter clearly, and when his argument was not followed. The criticisms of the audience were a great help in improving presentation. As a result our communications were so well given that the Physiological Society asked us to draw up recommendations which they printed and sent to all those who gave notice that they wished to give a communication. These said that communications must not be read, should be rehearsed, etc. After all, if someone expects the members of a large Society to listen to him, it is common courtesy for him to practise giving it until the communication is given as well as possible. Moreover the value of a communication is often judged by the way in which it is presented.

Acetylcholine in the heart.—Among other studies which continued over some years was one concerning the action of acetylcholine (ACh) in the heart. In 1946 there were various suggestions that acetylcholine was synthesized in the heart, and had a function which was in some way excitatory. We demonstrated the formation of acetylcholine (21) by perfusing the isolated rabbit heart through the aorta with Locke's solution containing physostigmine, and recirculating the fluid through the heart for 40 minutes. At the end of this time the fluid was collected and freeze-dried, and the residue was extracted with ethanol. The extract, when tested on cat blood pressure, frog heart, and frog rectus, was found to contain acetylcholine and also (22) an adrenaline-like substance. The hearts when beating spontaneously beat at 56 beats per min, and produced 0.26 μg ACh per heart per 40 min. They were also driven electrically at 210 beats per min, and then produced 1.32 μg ACh per heart per 40 min. Thus the amount of ACh formed was proportional to the rate of beating. We had no clue to its function.

In 1949 John R. Vane (23) was examining a new antimalarial compound, proguanil, and found that when isolated rabbit atria were exposed to its action, the contractions declined and finally stopped. But when acetylcholine was added to the bath, the contractions started again with great vigour, and stopped again when the ACh was removed. Later, A.K. Armitage (24) made a similar observation when he exposed isolated atria to quinidine; again the contractions declined and stopped, and again ACh caused them to start again. However, he also found that when quinidine

had caused the atria to stop, they started again when the amount of K^+ in the bath fluid was reduced from 5.6 mM to 1.4 mM. This suggested that the arrest of the atria by quinidine might be due to a diminished permeability of the cell membrane to K^+, so that in the presence of quinidine, the K^+, which must escape through the cell membrane after a contraction in order to repolarize the membrane, could not escape. It could escape when the gradient of K^+ concentration across the membrane was made steeper by reducing the external K^+. This suggestion at once explained the action of acetylcholine in restarting the contractions stopped by quinidine, because as Harris & Hutter (25) showed, acetylcholine increases the permeability of the cell membrane for K^+, so that the exit of K^+ is facilitated and repolarisation is once more achieved.

The effect of acetylcholine in restoring atrial contractions was also demonstrated (26) in circumstances in which the atria were left in the bath until they ceased to contract, perhaps after 70 hours. The arrest of the atria in this case was shown by Goodford (27) to be due to a steady fall in the intracellular K^+, so that the gradient from inside to outside again became insufficiently steep for repolarisation. But when acetylcholine was added, the permeability for K^+ was increased and repolarisation was once more achieved. The atria then resumed their contractions.

The most exciting results, however, were obtained by Jean Marshall & Vaughan Williams (28) who found that atria ceased to beat when they were cooled to a temperature below 20° C, and that their contractions could then be started by acetylcholine. Jean Marshall (29), when working at Johns Hopkins later, was able to explain this by showing that on cooling below 20° C the transmembrane potential gradually fell, and when it was below 60 mV impulses were no longer propagated, and therefore contractions ceased. However when acetylcholine was added, the transmembrane potential rose and propagation of impulses began again. The effect of acetylcholine in raising the transmembrane potential was again probably due (at least in part) to its effect in increasing the permeability of the membrane for K^+ and so facilitating repolarisation.

These results suggested that the transmembrane potential in rabbit atria could not be maintained below 20° C because it was normally maintained by a formation of acetylcholine which failed below 20° C. That this was so was demonstrated by observations on the activity of choline acetylase from rabbit atria. A. S. Milton (30) determined the Q_{10} for ranges of temperature between 37° C and 13° C. He found that from 37° C to 21° C the Q_{10} was approximately equal to 2.0 in every range of 4° C (i.e. from 37° C to 33° C, 33° to 29° C etc.) However between 21° C and 17° C it rose to 6.2, and between 17° C and 13° C it rose to 7.8. Thus synthesis of acetylcholine was greatly reduced below 20° C at the point where impulses were no longer propagated. This was a very satisfying conclusion, since it indicated that one purpose of the synthesis of acetylcholine in the atria was

to enable the transmembrane potential to be maintained at a sufficiently high level. It is interesting to note that whether acetylcholine is restarting atria which have stopped at a temperature below 20° C, or whether it is stopping atrial beats at a normal temperature, it acts in both cases by raising the transmembrane potential. I have given an account of this evidence because it is not well known, and because it led to a conclusion both unexpected and interesting. Thus an investigation (31) of isolated atria with the right vagus nerve attached showed that when cooled to a temperature at which the spontaneous contractions stopped, one pulse applied to the vagus would not cause the atria to start again, but two pulses would do so. When the contractions had continued for 5 minutes, then without change of temperature one pulse would arrest the contractions. After 5 minutes arrest, two pulses would start them again, and so on. These observations were re-

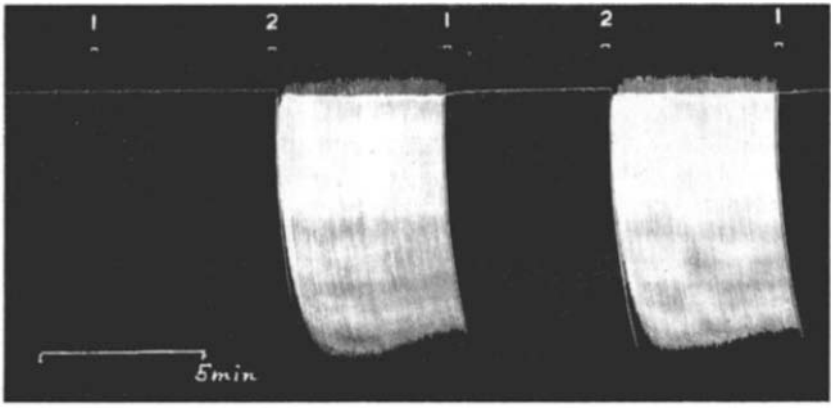

FIG. 1. Experiment showing the double action of the vagus on isolated rabbit atria. When the bath temperature fell to 17°C. the contractions stopped. At "1" a single pulse was applied to the vagus. It had no effect. Then at "2" two pulses at 1 sec interval were applied and the contractions then began. After 5 min, one pulse was applied to the vagus, and the contractions stopped. After 5 min more two pulses were applied and the contractions began again, to be arrested 5 min later by one pulse. These effects were repeated 30 times (31). (Reproduced by permission of the Editors of the *Journal of Physiology*.)

peated no less than 30 times in the course of an experiment. This fascinating situation, shown in Figure I, deserves further investigation.

The sympathetic transmitter and monoamine oxidase.—If a prefatory chapter such as this is to include confessions of failure, then it is essential that I should refer to papers which I published in 1952 and 1953 on the fate of the sympathetic transmitter, in which I put forward the view that the enzyme monoamine oxidase was the agent responsible for destroying noradrenaline. The evidence began with the finding that thyroid-feeding in rab-

bits diminished the amount of monoamine oxidase in the rabbit liver, and that thyroidectomy increased it. These observations were confirmed by others. But then it was demonstrated that the degeneration of sympathetic fibres in the nictitating membrane led to a fall in the amount of monoamine oxidase in the membrane, and before long I had reached the conclusion that this fall was responsible for the supersensitivity to noradrenaline which followed denervation. It was about two years before I faced the fact that the evidence from the catecholamine Corbasil (which has a $-CH_3$ group on the alpha carbon of the side chain) was sufficient in itself to show that the view was wrong. Denervation supersensitivity was present when Corbasil was injected, and yet it was not a substrate of monoamine oxidase. If a scientific worker can once make a mistake like that, and even give lectures about it, then any other ingenious ideas he may put forward must be eyed with the suspicion which is their due.

Work on auricular fibrillation.—The foregoing error was an error of insufficient thinking. But there was another error at that time made in relation to observations on isolated atria, and the effect of physostigmine upon them. I failed to realize that when atrial contractions were recorded by means of a lever working against a spring, the amplitude of the contractions on the drum depended on the rate at which the contractions occurred.

However, this work was the first stage of investigations of the action of physostigmine in the heart-lung preparation of the dog. These investigations proved to be very fruitful, and led us to a method of producing atrial fibrillation at will, and of stopping it at will. We applied electrodes to the tip of the right auricle. These were held in place by a spring, and did not pierce or damage the auricle. We also prepared a burette containing acetylcholine and could drive the solution into the superior vena cava at a slow uniform rate. When we stimulated the atria for 30 sec at a frequency of 14/sec, this caused fibrillation but the fibrillation returned to normal rhythm when the stimulation stopped. Similarly, when we infused the solution of acetylcholine into the blood at about 1 mg/min, the rate of the heart was slowed, but the rhythm was unaffected. However, when we applied the stimulation during the infusion of acetylcholine, then, when stimulation stopped, the atria continued to fibrillate as long as the infusion of acetylcholine continued. When the infusion was stopped, fibrillation gave place to flutter, and then to normal rhythm within one minute. We had hit on a means of producing fibrillation and of maintaining it for as long as we wished. We could allow it to continue for 90 minutes and then stop it by a turn of the burette tap. We could start it again ten times in one experiment (32).

We also studied ventricular fibrillation by perfusing isolated rabbit hearts through the aorta with Locke's solution. Stimulation of the ventricles at 25/sec for 3 minutes produced fibrillation which stopped when the stimulation stopped. This was at 32° C. But when the Locke's solution was modified in any way which would shorten the refractory period, then the fibrilla-

tion continued indefinitely after the stimulation stopped. Factors which had been shown to shorten the refractory period were oxygen lack, glucose lack, the presence of dinitrophenol or of sodium azide or of monoiodoacetate. That these factors shorten the refractory period is evidence that energy is required to maintain the normal refractory period which in cardiac muscle is very long (150 msec) compared with that in skeletal muscle (2 msec).

Why then should it be necessary to have a long refractory period in cardiac muscle? The answer is that a long refractory period protects the cardiac muscle against fibrillation in which the fibres are contracting asynchronously, and are therefore, like an asynchronous tug-of-war team, unable to exert force. The importance of the long refractory period is evident in the atria also, because acetylcholine greatly shortens the atrial refractory period. Fibrillation produced by initial stimulation of the atria during the infusion of acetylcholine is arrested when the infusion stops and the refractory period lengthens.

What then is the cause of fibrillation? If the fibres are stimulated at a high rate they fail to contract in synchrony, because the conduction of the impulse is slower and varies along different paths. When individual fibres do not contract synchronously, excitation spreads from one fibre which contracts to another which is resting. This excitation is effective when the refractory period is abnormally short and the fibre is excitable. Thus fibrillation occurs because the fibres stimulate one another (33).

Fibrillation may occur spontaneously in the perfused rabbit heart when glucose is absent for a long time, or if the K^+ in the perfusion fluid is reduced to 1.4 m*M*, which is one-quarter of normal. Both these changes reduce the refractory period. Dr. Carl Schmidt (34) in an Editorial made very favourable comments on these conclusions.

Ciliary movement and acetylcholine.—Because Gray (35) had shown that ciliary movement and cardiac contractions were similarly affected by a variety of factors, we began an investigation of the part played by acetylcholine in ciliary movement. The investigation was first carried out on the mucous membrane of the frog oesophagus, then on the rabbit trachea removed from the body (36), and finally on the gill plates of *Mytilus edulis* (37). In all three we measured the rate of particle transport and in the *Mytilus* gill plates we measured the rate of ciliary movement by a stroboflash as well. The particle transport was quickened in all three tissues by low concentrations of acetylcholine, and was depressed by high ones. We observed similar changes after applying physostigmine. The particle transport was depressed by *d*-tubocurarine. An extract of the mucous membrane of the rabbit trachea was found to contain acetylcholine, and the presence of both choline acetylase and of acetylcholinesterase was established. The gill plates of *Mytilus* were extracted, the extract purified and shown to contain acetylcholine by biological tests (parallel quantitative assays on three organs), and by chromatography. The presence of cholineacetylase and of

acetylcholinesterase was also determined (38).

The importance of these results was that the gill plates do not contain nervous tissue and we had demonstrated the function of acetylcholine as a local hormone in a situation in which nerves were absent.

A worker at University College, London (39) was unable to repeat our observations on the effect of acetylcholine and of *d*-tubocurarine on the frog oesophagus. Most of her observations were made using a phosphate-buffered Ringer's solution. Milton (40) discovered that, when phosphate was present, the inhibitory action of *d*-tubocurarine was no longer seen; however, the action was restored in the same preparation when bicarbonate replaced the phosphate. Few are aware that phosphate can interfere with tissue respiration. According to Alt (41) even the inhibition of the respiration of kidney and liver slices by cyanide was reduced from 98 per cent to 11 per cent when bicarbonate buffer was replaced by a phosphate buffer. Our results with acetylcholine and *d*-tubocurarine were independently confirmed by D'Arcy, Grimshaw, and Pickering.

Pharmacology today.—In this prefatory chapter I have tried to express my view of pharmacology by a series of examples of the discoveries which can be made by what some would describe as old-fashioned methods. I have chosen these examples because the discoveries were of importance, and in my view they reveal the harvest still to be reaped by investigations of this kind. It is a great pity that so many are attracted to molecular pharmacology, because they are leaving fields unexplored which are highly relevant to the study of pharmacology proper.

What then is pharmacology proper? It is first of all the study of the mode of action of substances used in the treatment of disease, and of course the discovery of new substances for use in disease. Departments of Pharmacology were set up about the turn of the century as a step towards making the practice of medicine more scientific. Medical students were to be taught how drugs act. The success of the Departments was not always obvious because some teachers of pharmacology dwelt too much on actions of drugs which had no relation to treatment, with the result that 30 or 40 years ago there were physicians who thought that pharmacology as a discipline for medical students was not worth while. With the rapid increase of potent medicinal agents, the need for a knowledge of their mode of action became more and more necessary. Nevertheless there are those who think that this kind of pharmacology can be taught by the physicians. The practising physician is, however, rarely acquainted with the mode of action of drugs, because he has no time to study it. He is too much involved with diagnosis and the other aspects of patient care.

What is it that these members of electoral bodies would prefer to see? Some of them speak of molecular pharmacology as being a more appropriate study. One wonders in the first place how a knowledge of molecular pharmacology will help the doctor to treat his patient, and in the second

place how much real understanding of molecular pharmacology the majority of students will gain from attending a course of lectures and practical classes. Molecular pharmacology is for the most part a subject only understood by those doing research in the field.

That simple methods can still break new ground and yield a rich harvest is well shown by the method of blood-bathed organs which has been lately introduced by J. R. Vane. He withdraws the blood from the carotid artery of an anaesthetized dog, and collects it in a reservoir from which it is pumped up above the dog to cascade over a series of isolated organs. The contractions of these organs are recorded on a series of drums. The blood is then returned to the dog. The organs over which the blood flows are chosen for their sensitivity to some particular substance. For example a strip of rat colon can be used to measure angiotensin in the blood, and the rectal caecum of a chicken can be used to measure adrenaline, distinguishing it from noradrenaline. They have used as many as 16 organs at once. This simple method has recently been used to demonstrate that angiotensin I is converted into the more active angiotensin II during passage through the lungs, and not, as previously thought, by the action of an enzyme in the blood. This method seems certain to yield much new information.

The release of noradrenaline.—Since 1959 when I reached the age limit of 67 for heads of departments in Oxford University, I have had the good fortune to spend my time in finding new tests for a hypothesis concerning the release of noradrenaline. The suggestion is that the sympathetic postganglionic fibre releases noradrenaline through the prior release of acetylcholine. Till now it has proved to be a wholly unacceptable view to many people on both sides of the Atlantic, largely, I think, because few have considered the details of the evidence since 1964. Yet, whether it is accepted or not, a hypothesis has a value, perhaps its main value, in stimulating investigations, and in challenging assumptions which have been accepted without question. Such an assumption is that the sympathetic impulse releases noradrenaline directly. Those who accept it must explain, for example, how a very low concentration of acetylcholine will abolish the response to stimulation of the postganglionic fibre, and prevent the release of noradrenaline. A large number of such observations have now been made which are not compatible with the view of a direct release. In time those who disagree with what has been proposed, will come to see that disagreement is not enough, and that they must, in their turn, explain these many observations. Observations concerning the transmission of nerve impulses should be one of the prime interests of pharmacologists. For me this continues to be an exciting subject which has filled the first nine years of retirement with activity.

The end.—Finally, would I choose a different career if I had my time again? After all it can be said that those who spend their lives in research leave little behind them. They are not sculptors or painters whose work may be preserved for hundreds of years. On the contrary, their research,

whether of their earlier years, or even of ten years ago, is forgotten while they are still alive. Nevertheless they have their consolations. I can think of no way of spending life with more satisfaction than it was spent in the Oxford department, where we met for a brief break at 11 a.m., later for lunch in the library, and for tea at 4:15. At one period there was a grand piano, and there were two who played Mozart, Beethoven and Haydn as duets. We were a community like a College Common Room, and at the same time all (or nearly all) very interested in our research. There was usually a good sprinkling of workers from abroad so that we felt ourselves an international group. Moreover, our work was mostly of the kind that required hand skill, and gave an opportunity for some artistry in execution. I have a letter from a one-time statesman (Lionel Curtis) saying that the Vice-Chancellor (at that time Richard Livingstone) had told him we were the happiest family in Oxford. We used to have the Vice-Chancellors to lunch; they were all Arts men, and we wanted them to see what the scientific life was like. Thus I am quite certain that I would choose to have it all over again.

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