HEINZ OTTO SCHILD

18 May 1906—15 June 1984

Elected F.R.S. 1966

BY

SIR JAMES BLACK, F.R.S.

FAMILY BACKGROUND

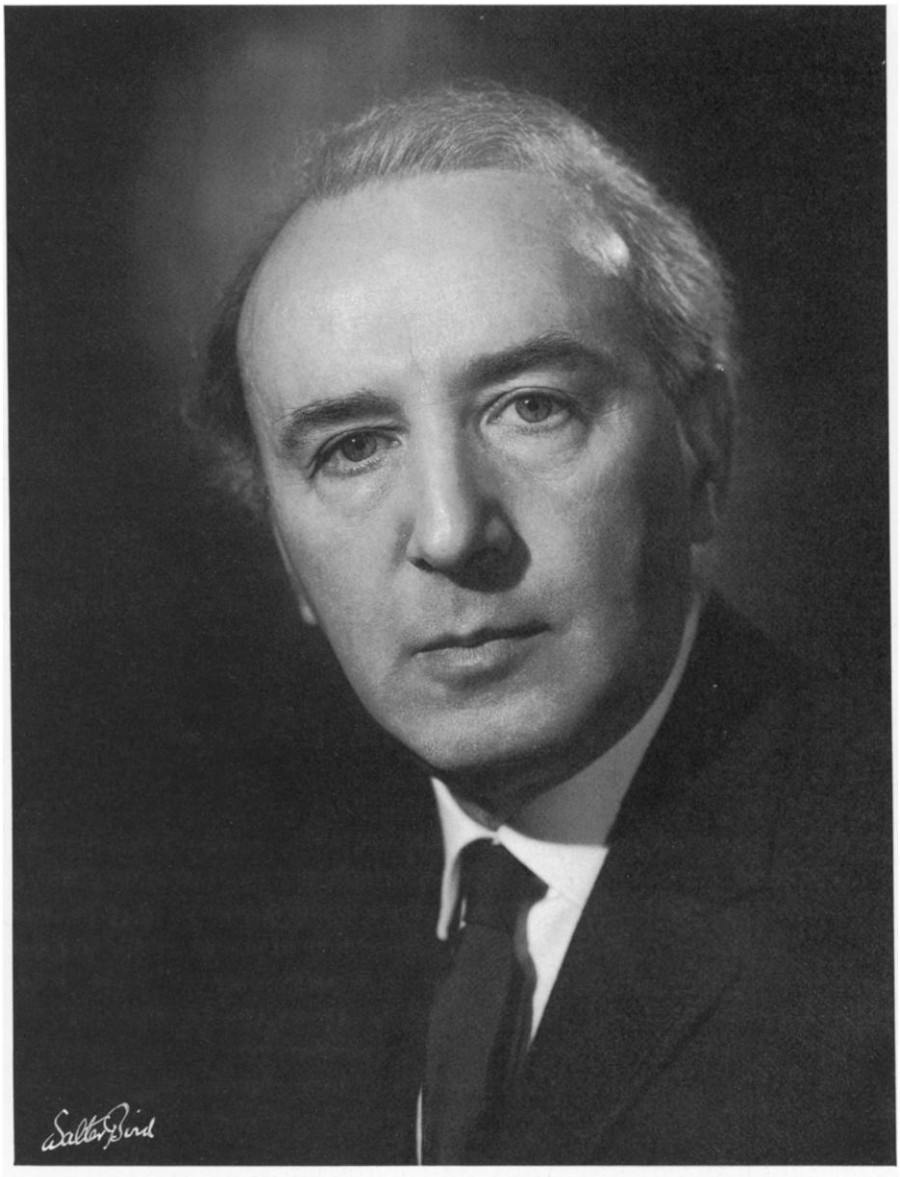
HEINZ SCHILD traced his family back to Eastern Europe. His grandfather, Adolph Schild, was a tailor. He married Caroline Mueller, a girl from Seret, his native town. They emigrated to Istanbul. They had seven children including Herman, Heinz's father. Herman Schild was born in Istanbul in 1872 and was educated by the 'Frères des Écoles Chretiennes'. However, he was a typical 19th-century agnostic with no interest at all in Jewish affairs. At the age of 16, he left Istanbul to avoid military service and got a job in Trieste. In 1897, he moved to Fiume (now Rijeka, Slovenia) where he joined a Hungarian navigation company called Adria, eventually becoming one of its directors. Herman Schild became an excellent linguist. He was an anglophile and so he was delighted when Heinz settled in England.

On his mother's side, Heinz' grandfather, Leopold Spiegel, was born in Austria. In 1877, he married Rosa Weiss from Budapest. They had seven children including Thekla, Heinz's mother. The family had settled down in Munich. They formed a typical bourgeois Jewish business family. However, at the time of the Nazi takeover, most members of the family emigrated to the US, Australia or South Africa. Hilda, Thekla's sister, however, was caught by the Nazis and died, perhaps by poisoning herself, on the way to a concentration camp.

Herman Schild and Thekla Spiegel married in Munich before beginning their family life in France. They had three children: Heinz, Marion and Herta.

ITALY 1906–1922

Heinz Schild was born in 1906 at Fiume, where (as he liked to point out) the eastern and western Roman Empires met. Fiume was a free port, lovely, open to the world. His parents lived on the water front in Palazzo Adria. The family spoke German at home but Heinz's first school was Italian. So, by the age of five, he had to learn two languages. Until the war, governesses gave the children private lessons in French, Italian, drawing, piano, dancing and, later, in English. He used to play football (not very well) on the Delta in Fiume. His school friends eventually elected him President of the football club, not for his football prowess but because his home had a marvellous loft for holding club meetings!



H Schmidt

When World War I broke out, life became difficult in Fiume. In 1915, Heinz was sent to grandmother Rosa in Munich. In 1917, he was sent to a Hungarian school in Budapest where food was still plentiful. In 1918, after the Armistice, he went back to Fiume, to an Italian elementary school. There he had his first introduction to pharmacology from studying the effects on Chentis, his botany teacher, of the bottle of wine always concealed in his desk.

Although still only 13, he developed a strong interest in Judaism and Zionism. In fact his interest in Zionism lasted until the Begin era switched him off. At school he learned Latin and Greek. With other bookish boys, he founded a book club, Bonorum Amicorum Bibliothecum, complete with a special stamp. Later on, they learned that it should have been Bibliotheca but they could not afford a new stamp! In retrospect, Heinz had only romantic and sentimental memories of these early years in Italy. He was good at school work, especially maths, but also with a love of literature, especially Dante. He remembered good friends and no bitter experiences.

The peaceful life in Fiume continued apparently undisturbed until 1921 when the ownership of Fiume, an important port, was disputed after the collapse of the Austria-Hungarian Habsburg monarchy. Heinz thought that his family's departure from Fiume was accelerated by a school essay he wrote full of sound international arguments, saying that the Madre Austriaca and Madre Italiana had both suffered during the War and should try to ease their differences. At school the teacher was a member of the Fascio, the Fascist Party, and the seditious essay was read out. The local fascists decided that Heinz's father should be purged from his responsible position. They rapidly moved to Munich.

These family upheavals meant that Heinz's education mainly consisted of learning languages, a fact which later on in life he came to appreciate. He strongly believed that languages open enormous horizons. Although naturally proficient in languages, he was generally more interested in scientific literature and mathematics. He also found that changing schools so many times gave him a sort of mental elasticity, a kind of skill in lateral thinking. On the other hand, he confided that all through his life he could feel huge gaps in his formal school education.

MUNICH 1922–1931

When Heinz was 16 he returned to the German Gymnasium in Munich. He found that the language transition from Italian to German was not too difficult. He read widely – Tolstoi in translation, but Stendhal in French, Dickens, Sterne, Swift in English and Thomas Mann, among many others, in German.

However, his time at Munich, especially at his boys-only school, was dominated by encounters with Nazism. One day (*ca.* 1924), a circular about a dancing class, with a big Hakenkreuz on the front page, went round. Heinz was stupefied. He confronted every pupil personally to ask them if they agreed with the anti-semitic emblem. All but a few did, explaining that this was not a personal move but that it just reflected their feelings for Nazis. Heinz was deeply hurt by the whole incident and broke contact with all the young Nazis at school.

During his late 'teens, his main interest was in the exact sciences, especially physics. Had it not been for family objections, he would have probably graduated in physics. The decision

to study medicine was a compromise made with his family after advice from the local doctor. The requirements of the Medical School at the University of Munich was the 'Matura' obtained after four years of elementary school and nine years of gymnasium. Despite a most irregular course of studies, Heinz was eligible. He studied medicine in Munich and Berlin. The German system allowed the students to spend one semester or more in other universities. So, he spent two winter semesters studying at the University of Berlin.

Munich Medical School was good, one of the best in Germany. However, the teaching was very old fashioned. Everything was centred around the lectures invariably given by the Professor. Experimental work was virtually non-existent. The preclinical curriculum lasted two years (first year programme: physics, chemistry, botany and zoology; second year programme: anatomy, physiology and biochemistry). From that period, the teachers which Heinz remembered best were Wien (Physics) and Willstaetter (Chemistry). Heinz attended Willstaetter's last lecture (he had to resign under Nazi pressure, although a Nobel Laureate) in which he explained in a dramatic fashion Rutherford's atom splitting and Bohr's atomic model; he left the auditorium under tremendous stamping by the students, lasting ten minutes, but he did not reappear. He remembered the anatomist Mollier, who was also a great actor. Whenever his teaching touched on his own experimental work he would dramatize the subject with a flow of tears, much to the delight of his pupils. Then there was Otto Frank, the great cardiovascular physiologist, who liked to talk about his experiences in England where he had been courted for his mathematical skills. Frank's original work on the length–tension relations of the frog heart (the 'law of the heart') was later extended to the mammalian heart by Starling. Much of his time in the preclinical course, Heinz followed wider interests and attended extra lectures such as physical chemistry with fajans, calculus with Caratheodory and French with Vossler, all at the expense of anatomy.

Heinz found his clinical training much better and more profitable. During clinical demonstrations in the auditorium, patients were actually shown to the whole class. Two students would be called down to the bedside to be questioned by the Professor. Of course, it was all very theatrical. For example, Schild remembered Sauerbruch, from Berlin, arriving in the morning with white tie and so forth, having obviously been revelling all night. The assistants jumped on to him, pulled off his coat, put on his white jacket and gloves . . .. Sauerbruch was then ready for the show. On the whole, Schild was impressed by his clinical teachers such as von Mueller, Romberg, His and Bumke, and by their demonstrations in internal medicine, paediatrics, psychiatry and ophthalmology. He believed that this impressive teaching allowed him to remember a great deal of medicine all his life.

Reflecting on the differences between medical education in Germany and England between the wars, Schild thought that preclinical teaching was much better in England but that the clinical instruction was better in Germany.

Having passed his Staatsexam (finals in medicine) in Munich (with a first), Schild spent some six months in the Schwabinger Krankenhaus in Munich as 'Medizinalpraktikant'. Konrad Dobriner, who later became an endocrinologist in New York, was his chief. He enjoyed that period of doctoring. He always felt that his medical training helped him as a pharmacologist, both professionally and generally, perhaps because medicine is such a human subject. However, he did not enjoy the inevitable personal involvement with patients,

and the intimacy he found difficult and exhausting.

The second part of the preregistration period had to be spent in physiology or Pharmacology. Schild rejected Physiology because he thought that Otto Frank, although a great physiologist in his day, was now too old and difficult. Fortunately, a friend introduced him in to Straub, whose lectures he had enjoyed. Straub took him in to his Pharmakologisches Institut in Munich. Straub, who was widely known for his potential theory of drug action, was the most important figure in German pharmacology at that time. The idea of the potential theory was that pharmacological action was due to fluxes so that the same effect of a drug could be seen during washout as during addition. The theory was so dominant at the time that virtually no pharmacological papers could be written without mentioning it, just like receptor theory in contemporary pharmacology. Incidentally, he invented the cannula (Straub's cannula) which Otto Loewi used to discover chemical neurotransmission.

The Pharmacology Institute was a fine building set in a beautiful garden. The caretaker lived in the basement. On the ground floor were the labs, quite well furnished and divided into two parts, physiology and chemical pharmacology. On the top floor was the professor's flat. Straub was an attractive bear of a man, always smoking a big cigar. He, like the rest of the department, was strongly anti-Nazi. From time to time, the whole lab was invited to a smart restaurant in Munich. Everyone found a big cigar at his dinner place, a sign that you were on your way to the top! Before going to the party, however, each member of the lab had to pay his homage to Madame Straub by going upstairs with a bunch of flowers and a visiting card, properly bent at the corner, but delivered to the maid without actually meeting Madame. Schild found the whole folderol highly amusing. In Straub's lab, Schild met a Swedish couple, Georg and Louise Kahlson. They became close friends for life. As they were to find out later on, they shared a spirit of mischievous irreverence.

Meanwhile, Straub fell ill and so Schild had to start working on his own. He tried to think out some interesting problem to tackle. All his life he was to recall the agony of trying to start research on his own. For instance, he tried to find a connection between blood dilution and diuresis, or to test whether insulin could be absorbed from the gastro-intestinal tract – all quite hopeless. This initial period of frustration made a deep impression on him and prevented him from ever trying the 'sink or swim' methods on his students. In the end, he was rescued by a colleague, von Werz, who asked to collaborate with him. This was Schild's first exposure to experimental pharmacology. They studied the absorption of ergotamine from the rectum, then a new drug used in obstetrics. They used the reversal of adrenaline's pressor response in spinal cats as the bioassay (1)*. In research our earliest successful experiments seem to mark us for life. There is no doubt that from then on Heinz Schild was imprinted with the problems of bioassay or pharmacological measurement. When Straub returned to the lab after his illness he put the whole lab on an interesting but limited problem – the effect of distension on intestinal motility in anaesthetized guinea pigs. In fact, this work became the subject of Schild's MD thesis. The most interesting result was the

* Numbers in this form refer to entries in the bibliography on the accompanying microfiche.

discovery of the powerful antagonism by intravenous magnesium of calcium-induced intestinal peristaltic contraction. Schild returned to this observation several times in his experimenting.

Schild found his time in Straub's Institute interesting mainly because of the personal contact with other research workers. It was the period of impending Nazi takeover but, in the laboratory, the atmosphere was intensely anti-Nazi. An anecdote about Georg Kahlson illustrates the mood of that time. In Munich there is a monument to the first Hitler putsch of 1924. Anyone passing the monument was expected to show respect by removing his hat. When Kahlson paused without doffing he was stopped by the guard. 'Why did you not take off your hat?' 'Why should I? No-one has taken their hat off to me!' 'Why should they – who are you?'. Kahlson showed his Swedish passport. The guard said 'If I came to Sweden and passed your monument of the unknown soldier, I would certainly salute'. So Kahlson said 'I hope you never come to Sweden'. Needless to say the Kahlsons had to leave Germany rapidly! Straub himself was evicted from his office by his chief technician who became the local Gauleiter. During the War, Kahlson's position even in Sweden was precarious because of his pro-British stand. He personally invited Niels Bohr who managed to escape from Denmark. After the War, Kahlson received several Allied decorations.

When working in Straub's laboratory, Schild, through a piece of great good luck, got in touch with Sir Henry Dale. Gremels, a colleague who had spent time in England, had been requested to translate a lecture to be given at the Wiesbaden Congress, by Dale, on vaso-depressor substances in tissues. However, Gremels fell ill and Schild was asked to take over the translation. Schild visited Dale in his hotel room. After a few minutes, unaware that Dale only took a single Fellow in his lab at any time, Schild had the gall to ask Sir Henry whether he could come and work with him in London. Sir Henry, probably taken by surprise, said yes. Schild kept in touch with him. In July 1932, Schild received a letter from Sir Henry: '. . . I am now in a position to consider your proposal, but before answering definitely, I must have a few more details. I presume that you have a Fellowship stipendium from some source, to provide for your personal support during your period of work in this country. Then, I should like to know as to the probable length of your stay. I am reluctant to accept workers here for a short period of only a few months, as, according to my experience, the result is usually to occupy a good deal of time for myself and my colleagues, making arrangements for visitors work, without any prospects of his being able to finish anything, and obtain any advantage proportionate to the efforts. I imagine, from what you told me, that you contemplate a somewhat longer stay; but I should like to have a definite indication on that point . . . I shall be pleased to ask authorization from the Medical Research Council to receive you here as a visitor, for a period beginning in October next, and hope that we shall find the arrangements of advantage to both sides.'

Early British period 1932–1938

Supported financially by his father, Schild arrived at the National Institute for Medical Research, Hampstead, in October 1932. He had three reasons for wanting to work in England. First, he knew from Gremels and other colleagues about the excellence of British physiology and pharmacology in general and of Dale's laboratory in particular compared

with the rather shaky state of those disciplines in Germany. Second, he had visited his father's English friends before and spoke English well. Third, the British political system appealed to him, even before Hitler's takeover in Germany.

The transition from Straub's lab to Dale's gave Schild the impression of leaving a dimly lit room to enter into a sunny open landscape. He immediately realized what an extraordinary favour it was to be allowed to work there. He was struck by its marvellous organization and, more important, by its powerful flow of ideas. The laboratory looked like a typical old-fashioned physiological laboratory, with the smoked drum as the mainstay. The laboratory, consisting of a single oblong room with two or three operating tables, was only part of the Institute, of which Dale was the overall director. The technical organization of the legendary F4 lab centred on Collison, the chief technician. Collison had to be informed the evening before of any planned experiments. Everything needed for the experiment would then be set out to perfection for the next morning. It was not advisable to change one's mind! Schild found it difficult to disentangle how many of the basic ideas emanated from Dale himself – who was kept rather occupied, though by no means exclusively, by his administrative duties – and how much from such outstanding co-workers as Feldberg and Gaddum. Schild vividly remembered these latter two first discussing and then performing the fundamental experiment on perfusion of the cat's superior cervical ganglion, an experiment which showed that acetylcholine had a ganglion-stimulating, nicotine-like action – an important link in the argument about acetylcholine's role as a neurotransmitter (157). Another landmark experiment he saw take place was Dale's experiment with Feldberg showing that the sweat glands in the cat's paw, although anatomically innervated by sympathetic nerves, were functionally cholinergic. Schild was hugely impressed by the way these investigators had to use Ringer solution souped up with extra potassium to get the leech assay to work and then Dale's famous challenge 'If they don't believe it, let them repeat the experiment!'. The atmosphere in the laboratory was always friendly and exciting. Schild was amazed that Gaddum spent a great deal of time showing him round the Institute and introducing him to Harold King, Rosenheim and Dudley. Contacts with other laboratories were close, their advice and help often requested and was always well received. Like other members of the team, Schild believed that in that environment scientific problems were self-generating and that important questions worth researching into were never lacking.

During that year, Schild's investigations turned out to be forerunners to developments several years later. Dale suggested a problem that nearly led to the discovery of the natural occurrence of noradrenaline in the body. Here was the problem. Albert Szent-Gyorgi and co-workers had claimed that they had found in adrenal extracts a precursor of adrenaline which had the same colorimetric activity but ten times its biological activity. This principle was called 'novadrenine'. Having modified the colorimetric assay procedure, Schild repeated the experiments and came to the conclusion that the Hungarian workers had overlooked the importance of pH for colorimetric estimations of adrenaline. Under Schild's conditions, most of the discrepancies between biological (spinal cat) and colorimetric assay disappeared. Nevertheless, even the most careful experiments showed the 40% superiority of the biological assay of the adrenal medullary extract over the colorimetric estimate (3). When writing the paper with Dale, Schild considered various possible explanations for the

discrepancies but it did not occur to him, and apparently not to Dale, that it could have been due to the medullary extracts containing both adrenaline and noradrenaline as was proved a few years later by Holtz. During a visit to Dale's laboratory, Szent-Gyorgi was told of Schild's findings and he exclaimed: 'Here is the murderer of novadrenine.', and laughed heartily. Schild learned then that geniuses can make mistakes and acknowledge them.

In the course of investigating the colorimetric assay of adrenaline, a fluorescence assay for adrenaline was developed with Gaddum (4). Barker, Eastland and Evers had found that various reagents plus alkali make adrenaline fluorescent. Gaddum and Schild found that adrenaline, oxygen and alkali were the only ingredients required to produce a transient fluorescence. This observation provided a quantitative measurement for adrenaline. The new method was more than ten times as sensitive (1 in 200 million) as existing colorimetric tests. Noradrenaline gave very much less fluorescence. Schild tried to demonstrate the occurrence of adrenaline in suprarenal venous blood by this test but did not succeed because of interference from a blue fluorescence generated by the serum. The fluorescence method was later perfected by Danish workers and became the standard method of adrenaline assay.

In the meantime, Schild did an experiment with Feldberg (Feldberg's idea) showing that the adrenal medulla contained acetylcholine and the cortex mainly choline (5). In another set of experiments, Schild tried to purify substance P, recently discovered by von Euler and Gaddum. The paper led to the introduction of certain general principles by which tissue extracts could be analysed. For example, histamine-like or adenosine-like activity could be separated by boiling extracts in strong acid or alkali, respectively (6). Similar procedures later led to the Barsoum, Gaddum and Code methods for estimating histamine in tissues.

Schild considered Dale as a personality of tremendous force and as a very passionate investigator. Dale had total faith in his own findings. Feldberg said that Dale worked like an astronomer. He prepared the investigation perfectly, and then did just one or two experiments that allowed him to address properly the next issue. Schild has remarked that Dale's conversation was outstandingly lucid and informative. Talking to him on a scientific subject was like wandering through a sunlit landscape in which every detail is distinctly delineated; the clarity, intensity and confidence of his thought adding to its impact. Dale had achieved the highest honours. Yet he remained personally completely simple, approachable, interested in people, and kind. (131)

Although Schild did not initially intend to settle down in England, as he considered himself a doctor and was not allowed to practice without a British medical qualification, he decided to stay in Britain (in 1933) when the Nazis came to power. As Dale had provided him with hospitality for one year, Schild started looking for a job somewhere else in Britain. He had been told about Professor I. de Burgh Daly (generally regarded as an eminent physiologist) who had just taken up the Chair in Physiology at Edinburgh. Schild wrote and asked to join him. De Burgh Daly generously agreed, although he had just moved in to his new labs. De Burgh Daly introduced Schild to the problem of histamine and anaphylaxis, a problem which was to become one of Schild's main interests throughout his life. He spent three years in the Physiology Department working on the histamine-anaphylaxis problem. He was working mainly on his own and completed the work for his Ph.D. thesis. During this time he was funded partly from an Asthma Research Council grant to Daly and partly

from his own resources.

For his first investigation, using some sophisticated ventilation apparatus constructed by de Burgh Daly, he was able to confirm Feldberg's finding that histamine was released from perfused guinea-pig lung in anaphylaxis (8). In a second set of experiments, he showed that the isolated guinea-pig uterus became gradually desensitized when exposed to a supramaximal concentration of histamine. After wash-out, although the tissue now failed to respond to histamine it still responded to antigen, suggesting the presence of other active substances (9). Those results were later confirmed by Brocklehurst who discovered the Slow Reacting Substance of Anaphylaxis (SRSA) at University College. Schild then made two important biochemical observations about histamine. He showed that histaminase destroyed the anaphylactic 'shock substance', providing further evidence that it was histamine (7). Then he showed that histamine released in anaphylaxis cannot be derived by decarboxylation of histidine residues in the antigen, as had been suggested, because the amount of histidine in a minimum eliciting dose of egg albumin was quite insufficient to account for the released histamine (10). Schild then studied the mechanism of histamine release, showing that it is not an indirect consequence of smooth muscle contraction because barium and all the other 'anaphylactoid' agents failed to release histamine (11). An interesting point in this paper was the discovery of a dual action of adrenaline. He showed that adrenaline had two inhibitory actions in anaphylactic shock: it relaxed the bronchial muscle directly and it also diminished the release of histamine. Schild, here, was ahead of his time. Much later on this observation was taken up and elaborated with Assem (119). Meanwhile, a new method of demonstrating histamine release by diffusion from cut tissues was developed by Schild (discovered independently by Ungar in Paris and later developed into the quantitive 'shocked-lung' technique by Mongar). Schild showed that most guinea-pig tissues released histamine in anaphylaxis (aorta most, gut least) (13). He also studied the kinetics of histamine release and the influence of cooling (later elaborated with Mongar). By intermittent cooling, the histamine release reaction was shown to be at least 50% complete during the first 30 seconds following application of the antigen. In addition, the 'effectiveness' of released histamine was quantitatively not different when compared to added histamine (16).

En passant, Schild demonstrated that adrenaline reversal in the cat could be produced by administration of oestrogen and progesterone. Sadly, this observation was never followed up.

Schild considered his time in the Physiology Department at Edinburgh as a formative period where he learned to work on his own. During his last year in Edinburgh, he held a position of assistant in the Pharmacology Department. This was his first full-time salaried position. The Professor of Pharmacology was A.J. Clark. Although he did not actually work with Clark, he was influenced by his thinking and translated into German several chapters of his book on General Pharmacology. His year with Clark was notable for two events – his collaboration with J.M. Robson and his first paper to the British Pharmacological Society. The paper, which was unpublished, dealt with a theme dear to Schild's heart. From his early interest in the physical sciences he always retained a strong interest in chemistry and its relation to pharmacology. So, during his work on histamine and anaphylaxis he had

developed the idea that he wanted to find a specific histamine antagonist – an 'atropine' for histamine. In the event he was unable to generate any chemical collaboration. However, he had found that tryptamine, though not selective for histamine, did show quantitative antagonism to histamine over a wide range of concentration. Given chemical support, Schild might very well have anticipated Bovet's discovery of the anti-histamine drugs.

In his collaboration with Robson, the start of a life-long friendship, Schild worked on the effects of drugs on the uterus in situ in anaesthetized cats. The original idea was to try to evoke secretion of oxytocin by electrical stimulus of the posterior pituitary. They failed. On reflection Schild thought that their technique was too crude; in fact Harris (1939) succeeded soon afterwards by using tiny implanted induction coils. Even so the collaboration produced very interesting observations on the interaction between ovarian hormones and posterior pituitary hormones when acting on the uterus *in vivo* and *in vitro* (12, 14, 15). A problem which intrigued them was that vasopressin, which in vivo inhibited the uterus of cats treated with ovarian hormones, had no effect on the uterus *in vitro*. However, they showed convincingly that vasopressin-induced vasoconstriction was not the explanation. Schild was to return to uterine pharmacology much later on (71), but this time to assess oxytocic activity in women.

He enjoyed his time in Edinburgh and would have been happy to stay there. However, when Gaddum returned from Cairo in 1937 to fill the Chair at University College, London, he invited Schild to join him as a demonstrator. Schild accepted. The team was expanded to include his old friend Georg Kahlson plus David Smyth and Rod Gregory from the College. Soon afterwards, Gaddum was offered the Chair at the School of Pharmacy and decided to leave. Schild, although invited by Gaddum to join him, preferred to stay at University College.

Before leaving, Gaddum suggested a histamine problem which had cropped up during his research in Cairo. The problem was the alleged increase in blood histamine during muscular exercise and reactive hyperaemia. Schild and his colleagues spent a lot of time on this problem but failed to detect any change in blood histamine levels. However, in research, no serious investigation is ever entirely sterile; the work led, later on, to two new lines of research which turned out to be highly fruitful; the discovery of histamine-releasing agents and major contributions by Schild to quantitative pharmacology.

Although Gaddum remained a friend for life this was to be the end of their research collaboration. For Schild, Jack Gaddum had been the scientist who – after Dale – had influenced his way of thinking the most. Gaddum was an excellent mathematician who would not rest until he had personally solved the problem. Occasionally, Schild thought, he got the wrong end of the stick by being too logical. Nevertheless, he found Gaddum's analytical thinking particularly stimulating. Gaddum believed in doing the minimum number of experiments that was statistically required. In Schild's opinion this was a weakness because true randomization is seldom achieved. However, Schild found Gaddum's collaboration and supervision highly beneficial, especially in the fields of bioassay. As a man, Schild found Gaddum emotional, creative and very straightforward.

Soon after Schild got back to London in 1937, he met, and later married, Mireille Madeline

Haquin, a lovely French girl from Paris. They had met on a walking tour along the North Downs Way; walking was one of Schild's favourite pastimes.

INTERNMENT 1939–1940

In 1939, Schild was still an Italian citizen. When war with Italy broke out, in May 1940, he was interned in the Isle of Man. Feldberg thought it was a scandal and immediately contacted Esther Simpson, Secretary of the Society for the Protection of Science and Learning. The Society then approached Winton, Clark, de Burgh Daly, Gaddum and Dale for references. They all supported him enthusiastically.

A particularly distressing event occurred in August 1940. Mrs Schild was officially informed by the Home Office that Dr Schild was sunk on a ship sailing to Canada:

> Madam, it is with deep regret that the Secretary of State directs me to inform you that since the name of Dr Heinz Otto Schild appears on the list as sailing on the Arandora Star, on 30 June 1940, and has not been subsequently recorded on the embarkation list of internees, who have left this country for Canada or Australia, or among those detained in internment camps in this country, it must be presumed missing and probably lost.

Mercifully she soon learned that Heinz was safe in the Palace Camp, Douglas, Isle of Man.

For Schild, internment was an interesting experience. As a doctor, he was assigned to the camp medical department led by a local Isle of Man doctor. One day Schild was working in the camp hospital and a soldier told him to go upstairs and fetch a patient. Schild refused, pleading that he was busy at the hospital. Now in these camps every soldier had to be obeyed by a mere inmate. So, Schild was summoned at once to the Camp Commander where he tried to explain. He was curtly interrupted: '168 hours!' Schild trotted off under escort behind the barbed wire. Schild remembered that solitary confinement as a great experience for meditation and a wonderful opportunity to concentrate on deep thoughts! During the confinement, Schild got a cigarette from a fellow prisoner. He had just finished smoking it when the sergeant in charge entered his jail. The sergeant shouted at once: 'Did you smoke here?' Schild realized that he had better confess. The sergeant shouted even more loudly: 'Don't you know that smoking is strictly forbidden?' Then, he came near Schild and said softly: 'You bloody fool, if you want a cigarette, don't ask other prisoners. Ask me!' From that day, they got on on excellent terms.

During internment, Schild got some news from time to time from his wife and baby. They had been evacuated to Bangor with some relatives. He also got letters from friends such as A.V. Hill. In his letter Hill emotionally expressed how wrong it was that refugees from Nazidom should be interned . . . in such desperate times, those letters made a deep impression on Schild.

During the Autumn of 1940, bombs drove the Department of Pharmacology out of the building in London. The Medical School had to be evacuated to a large country house outside London at Leatherhead, Surrey. As Head of the Department of Pharmacology, Frank Winton converted the country house into laboratories and other requirements of a Medical School. The teaching of Pharmacology was going by default. Therefore it was imperative that Schild return to his post as soon as possible. Thus, Winton and the Provost of University College, Sir Allen Mawer, wrote to the Home Office urging upon them the importance of securing

Schild's release. In addition, Schild's claims for release were supported by heads of all departments in which he had worked, starting with Sir Henry Dale, by the Royal Society and by the Committee of Vice-Chancellors of the British Universities.

In the meantime, however, Schild had applied for admission to the Pioneers. When Winton heard about this, he immediately got in touch with Professor Hill. He also wrote to Schild to tell him that he urgently wanted him at University College for the teaching of pharmacology and that he hoped that the Pioneers would reject him on that account. Around Christmas, Schild was called before an alien's tribunal, and accepted to go into the Pioneers. Fortunately, other events overtook that decision. On 2 January 1941, Mrs Schild received a telegram from Winton: 'Release authorized – congratulations', and a letter from A.V. Hill ending with the following words: 'Professor Winton will be glad to have your husband's help next term in his classes'. Thus, Schild was sent to University College where he lived in close contact with his students and managed to continue research quite successfully with Rod Gregory in transformed stables. For instance, his papers on the 'crush-kidney' syndrome are a piece of war-time research (17, 19).

After the War, Schild applied for British citizenship. On 4 June 1948, he received the following note from Sir Henry Dale: 'Welcome to the new British citizen'.

Research activities 1941–1973

As Schild has remarked, his own original experimental work and pharmacological thinking actually started during the War and several lines of research were developed over the years. Although his various research interests throughout his life may seem quite diverse, he saw them relate to a central theme. In 1979 (157), he wrote 'Retrospectively, I see the concept of autopharmacology – i.e. regulation of body function by pharmacodynamic agents produced in the body – as the central plank of my own pharmacological thinking, although this has developed in unexpected directions'. These various research activities can be grouped into six main themes: histamine and anaphylaxis; autosensitization and delayed hypersensitivity; development of bioassays; quantitative analysis of drug receptor interactions and their antagonists; functional analysis of drug-receptor interactions; and clinical pharmacology.

1. Histamine and anaphylaxis

Before leaving University College, Gaddum had suggested that further investigation of histamine in the blood would be worthwhile. Schild decided to pursue the project. This 'autopharmacological' subject followed him throughout his career and provided the opportunity for a long and fruitful collaboration with his friend and colleague Jack Mongar. Always in the background of his mind were his earlier observations in Edinburgh on histamine release and anaphylactic shock (11).

Histamine-releasing agents

Anrep, based in Cairo, had claimed that muscular activity raised histamine levels. Therefore he expected that muscular paralysis would lower blood histamine levels. However, Anrep had found that after muscular paralysis had been produced by tubocurarine,

a dramatic increase in blood histamine concentration occurred rather than the expected decrease. In contrast, tetraethylammonium ions produced no changes in histamine concentration in spite of causing neuro-muscular blockade. Although Gaddum and his team failed to confirm Anrep's exercise claim they were intrigued by his second claim about the effects of muscular paralysis.

Schild discovered that while histamine was released by strychnine methiodide, the quaternary ammonium salt and a powerful curarizing agent (made for him by Ing), strychnine base, the tertiary amine, which had practically no curarizing action, nevertheless had powerful histamine-releasing activity. So, in addition to the prototype, tubocurarine, there seemed to exist a whole class of substances able to cause histamine release and that this effect was independent of the muscle-relaxing properties. Schild continued working on this subject on and off for several years, although he published only a short note with Gregory in 1947 (22).

When Rocha e Silva visited him in 1949, Schild returned once again to the curare-histamine problem. Histamine release by tubocurarine was demonstrated to be limited by the rate of diffusion in the tissues. They measured the apparent diffusion coefficient for histamine and found it to be only slightly greater than for urea, another simple base. These were very elegant experiments. Schild also showed that histamine was released by the simplest amine, ammonia, but exclusively by the base, not by the ammonium ion (25).

Anaphylactic reaction of smooth muscle

Another issue addressed by Schild was the following: could histamine release alone account for the anaphylactic reaction of smooth muscle? Years before, he had noticed that an isolated guinea-pig uterus sensitized to ovalbumin could be treated with large doses of histamine until it failed to respond to it by contraction (9). However, the addition of the antigen, ovalbumin, to the bath at this stage caused a maximal contraction, suggesting that the anaphylactic response of the uterus could not be fully explained in terms of histamine. These experiments gave an early indication that factors other than histamine may be involved in the anaphylactic reaction of smooth muscle (27). In these studies, as in many others, Schild is remarkable in the way he kept an open-minded approach to his experiments. In a later paper (39) he wrote: '. . . possibly the major objection to attributing anaphylaxis and allergy to so simple a mechanism as histamine release lies in the fact that anti-histamine drugs are not very active against allergic reactions involving plain muscle.' On the other hand the importance of histamine release in anaphylaxis and the profound ignorance about the mechanisms of its control were shown by the parallelism between histamine release by antigen and chemical release by compound 48/80 and by the absence of a correlation between the histamine content of a tissue and the fraction released. Particularly important was the unexplained observation that pretreatment with 48/80 to release histamine did not suppress a subsequent anaphylactic reaction.

Histamine and bronchial asthma

An opportunity to deal with the role of histamine in human bronchial asthma arose

accidentally through observations made by Herxheimer on a patient who, in spirometer tests, reacted to pollen inhalation with an asthmatic attack. Subsequently, this patient required surgical removal of a lobe of his lung because of bronchiectasis. Hawkins prepared isolated bronchial chains from the excised lobe (157). The bronchial chain preparations of this patient reacted like the classical preparation of the isolated sensitized guinea-pig uterus, producing a powerful bronchoconstriction with the specific antigen, pollen extract, followed by desensitization to pollen but not to histamine (34, 39). Histamine release from the patient's excised lungs by pollen extract was demonstrated by biological assay. This was the first demonstration of histamine release in human allergy. Of particular interest was the so-called 'antihistamine paradox'. While the isolated bronchial chain responded to histamine added to the bath by a contraction that was readily antagonizable by antihistamines (e.g. mepyramine), bronchial-chain contractions elicited by pollen were not antagonizable by antihistamines unless extremely high concentrations were applied, supporting the clinical finding that antihistamines are ineffective in bronchial asthma (34, 39).

Inhibition of histamine release by sympathomimetic amines

During his period in Edinburgh Schild had found that adrenaline inhibited antigen-induced histamine release in isolated, sensitized, Ringer-perfused guinea-pig lungs. Adrenaline was added to the perfusate immediately preceding addition of the antigen and was then oxidized to enable bioassay of any released histamine to be carried out. It was found that adrenaline produced a statistically significant reduction of anaphylactic histamine release (11, 157).

Over 30 years later, Schild resurrected this problem in his collaboration with E.S.K. Assem. They confirmed the old observation in a new context; that adrenaline and related catecholamines, at concentrations less than 1/1000 of those of the most active inhibitors previously reported, could prevent antigen-induced histamine release in human passively sensitized lung tissue (119, 138). The order of activity of the catecholamines (isoprenaline most active, concentrations between 5×10^{-12}M to 5×10^{-9}M) suggested an action on beta-receptors, although, curiously, dopamine was also remarkably active. Further studies by Assem and Schild showed that the sympathomimetic amines could also inhibit histamine release in both actively and passively sensitized guinea-pig lung (121, 138). The isoprenaline effects were shown to be antagonized by adrenaline beta-receptor blocking agents (propranolol, practolol, butoxamine) (135). In addition, the methylxanthines, theophylline and caffeine, were found to produce similar effects on human and guinea-pig lungs, but they were less potent (inhibitory concentration range of 10^{-7} to 10^{-6}M) (121). Assem and Schild had the idea that low concentrations of circulating adrenaline in human beings might provide an auto-regulatory mechanism by which bronchial allergy was controlled, and that when beta-adrenergic receptor stimulants was employed in bronchial asthma, they might exert a dual effect, part bronchodilator and part anti-anaphylactic (136, 139).

Potentiation by histaminase inhibitors

In another set of experiments, Schild and his colleagues demonstrated that semicarbazide, carbonyl reagents, diamines, guanidine and imidazole derivatives shared the property of

being able to potentiate the effects of histamine on the isolated guinea-pig ileum, uterus and tracheal chain (36, 47, 48). Schild was resurrecting work he had done many years before in Edinburgh (7, 8). They showed that these compounds also shared the property of being inhibitors of histaminase. Indeed all the histaminase inhibitors they investigated also potentiated the effects of histamine. Moreover, they showed that the potentiating activities of different compounds correlated with their inhibiting activities. The tissues showing the greatest potentiation (trachea) also contained the most histaminase. The final piece of evidence linking histaminase inhibition with potentiation was that histamine-like compounds which are not substrates for histaminase are not potentiated. Schild here used the pA (see later) methodology to show that these substances, pyridylethylamine and pyrazolethylamine, did appear to be acting at the same receptors as were activated by histamine. It was also typical of Schild's quantitative approach to pharmacology that he tried to calculate whether the histaminase activity, measured by half-times for destruction of histamine, was sufficient to account for the degree of potentiation. In spite of having to make some whopping assumptions he nevertheless concluded that ' . . . it appears that the enzyme activity is of the right order for the observed potentiations . . . '

Soluble antigen-antibody complexes

Ever since Dale's early studies, anaphylaxis was generally assumed to be due to antibody becoming fixed to cells. On subsequent exposure to antigen, the combination with cell-bound antibody was supposed to be the trigger for the events leading to anaphylaxis. In this model, circulating antibody was deemed to have a neutralizing, protective, role in mopping up some of the antigen. However, several reports in the 1950s were pointing to a different story, that soluble antigen–antibody complexes could, in fact, trigger anaphylaxis. Broder, a Medical Research Council Fellow from Toronto, came to work with Schild and brought with him some soluble antigen-antibody complexes which had been made in antigen excess. They planned to re-examine the whole problem (157).

Using a vascularly-perfused, isolated, unsensitized, guinea-pig lung preparation which had been developed with Arunlakshana (75), Schild and Broder (99, 103) showed that soluble antigen-antibody (Ag–Ab) complexes produced a bronchoconstrictor response which was associated with the release of both histamine and SRSA, Brocklehurst's 'slow reacting substance of anaphylaxis'. Now it was well-known that certain macromolecules could produce bronchoconstriction in guinea pigs which was not due to anaphylaxis. A crucial observation was that this soluble-complex activity could be inhibited by normal serum. Normal serum is capable of inhibiting passive anaphylactic sensitization in vitro (79) but not the macromolecular bronchoconstriction. This inhibitory activity in passive anaphylaxis was believed to be due to competition between antibody and non-specific serum globulins for common sites. Using a similar argument, Broder and Schild proposed that the soluble Ag–Ab complexes had to bind to cell-surface sites before the anaphylactic process could be induced.

Looking back on this work, Schild (157) suggested that 'Our interpretation of these effects was that these soluble Ag–Ab complexes might represent a foreshortened model of the anaphylactic reaction. In passive anaphylactic sensitization the normal sequence of events

is likely to be as follows: (i) attachment of antibody to cell receptors; (ii) attachment of antigen to antibody; and (iii) intracellular reactions leading to transmitter release. With soluble complexes, stage 2 occurs *in vitro*. Stage 1 is foreshortened and is immediately followed by stage 3.' The clear conclusion was that, in the presence of antigen excess (presumably the usual clinical situation), combination with antibody in the plasma provided no protection in anaphylaxis.

Mechanism of anaphylaxis

For scientists of the highest quality, a major problem is loneliness. These people, Schild was one of them, live within the constricting bounds of their own dreams, thoughts, analyses, imaginings, speculations and, as Schild would always say in conversation, ' . . . and so on and so forth . . . ' A very lucky break for these people is when they meet up with someone who is complementary to them in their scientific strategies. Schild's collaboration with Jack Mongar was, I believe, one of these luckily fruitful encounters at University College London. The agenda they set themselves was to work out the mechanisms of anaphylaxis. Schild, I suspect (because I was privileged to get to know a little about both of them) brought to the collaboration 20 years of experience in the field and a natural attitude of caution and tentativeness; Mongar would have brought vitality, irrepressible enthusiasm and optimism plus the impeccable experimenting practices of someone obsessed with the search for 'truth'. More importantly, Jack Mongar brought a biochemical technology to the party. Between them they wrote over 20 papers between 1950 and 1960 analysing the mechanisms of anaphylaxis.

In a first set of experiments, Schild and Mongar found that, when guinea-pig lungs were homogenized in cold sucrose solution, the histamine was concentrated in the mitochondrial fraction. The histamine-containing particles are unstable and readily lose histamine. The process cannot be reversed by adding histamine. Histamine was released by chemical releasers such as octylamine and 48/80 from these particles. In contrast, histamine release by antigen required the presence of intact, sensitized, cells (51, 56).

Schild and Mongar then turned their attention to the inhibition of the anaphylactic reaction. Haptens, which interfere with the antigen-antibody combination, and antihistamines, had been thoroughly studied and found to be relatively ineffective. Schild (11) had studied the effects of adrenaline as an anaphylactic inhibitor many years before. This time they tackled the problem more specifically by trying to interfere with the metabolic processes involved in anaphylaxis. They found that many metabolic inhibitors and anti-inflammatory drugs, particularly phenylbutazone, were effective. They summed up their findings, 'The conclusion that the anaphylactic reaction requires a functioning cell is in accord with the finding that all substances which inhibit histamine release in anaphylaxis inhibit muscular activity and oxygen consumption. In general, the concentrations which inhibit contractility and oxygen consumption are the same order as those which inhibit histamine release by antigen' (64).

Next, they showed that calcium was of unique importance for the anaphylactic histamine release mechanism in guinea-pig lung. Omission of ionized calcium from the Ringer solution greatly diminished histamine release. Addition of a chelating agent abolished

histamine release. In contrast, the effect of a typical organic histamine releaser, octylamine, was undiminished by lack of calcium. In addition, calcium and pH interacted in a complex way. A low pH of 6.2 (bicarbonate buffer) produced total inhibition of histamine release but this could be counteracted by a tenfold increase of calcium ions. Conversely at a pH of 7.8 very little calcium was required for optimal effects. Possibly the anaphylactic mechanism required bound calcium and the binding might decrease with pH (66, 69). These studies were important because their previous attempts to inhibit anaphylaxis threw doubt on the possibility of discovering a selective inhibitor but 'the fact that calcium lack and acid pH inhibit anaphylaxis but not oxygen consumption shows that it is possible to block the anaphylactic reaction without affecting the cell as a whole' (69). Mongar later suggested that anaphylactic histamine release may be initiated by the entry of calcium into mast cells. There is evidence that the anti-asthmatic drug, cromoglycate, may produce its action by inhibiting this calcium entry. Unfortunately, cromoglycate only acts when delivered locally. Lack of calcium also affected desensitization. In the absence of calcium, desensitization was slowed down but not completely prevented. Conversely, desensitization of guinea-pig ileum by histamine was significantly decreased in the presence of a high concentration of calcium in the desensitizing solution (146). There was no evidence that other essential ions were required for the anaphylactic mechanism but the participation of a trace of magnesium could not be excluded (113).

Twenty years previously, Schild (16) had shown that temperature was an important factor in the anaphylactic histamine release. Schild and Mongar (65) now made a comprehensive study of the phenomenon. A slight elevation above normal body temperature (to 45°C for a few minutes) caused inactivation of the anaphylactic mechanism when tested afterwards at 31°C. A heat-labile component, which appeared to be an intracellular protein, denaturated progressively at temperatures between 42.5°C and 45°C. This component was not serum complement which requires temperatures of 54–56°C for inactivation. The inactivation rate had a high temperature coefficient, being doubled for each degree rise in temperature. The preparations appeared to retain their viability after exposure to high temperatures since chopped guinea-pig lung maintained its oxygen consumption and guinea-pig smooth muscle its response to histamine after exposure to 45°C (65). At low temperatures, the anaphylactic mechanism was not inactivated but reversibly inhibited. A sensitized preparation which was brought temporarily to 0°C and then returned to 37°C gave normal anaphylactic responses but if the antigen was applied at 17°C anaphylactic histamine release was inhibited. Thus, at 17°C, some processes were slowed down but other processes leading to histamine release were completely blocked. At 0°C, both histamine release and desensitization were completely blocked (65). Schild and Mongar were now building a model of anaphylaxis where Ag–Ab combination activates an enzyme system which catalyses reactions leading to histamine release. 'The activated enzyme is short-lived and in the normal course of events becomes rapidly inactivated' (65). They were still searching for selective inhibition.

Having established that anaphylaxis involved the activation of a labile enzyme, they now tried to characterize it. They tried to assess the role of SH and S—S groups in the reaction. Reagents which inactivate sulphydryl groups, such as N-ethylmaleimide, completely inhibited histamine release. Agents which reduce S—S bonds, such as sodium sulphite,

inhibited release. The effects were fully reversible and lower concentrations of reagents produced moderate potentiation. They suggested that the effects of anoxia are due to a reduction in S–S bonds and not to an essential role of O_2 per se in the reaction.

There was no disagreement in the 1950s that the first stage in anaphylaxis involved binding of antibody to cell receptors – passive sensitization. Passive sensitization can take place in vitro using chopped guinea-pig lung (65). Schild and Mongar systematically studied the mechanism of passive sensitization (74, 79). They demonstrated that passive sensitization increased with time of incubation and became maximal in two to four hours at 38°C. Passive sensitization had a high temperature coefficient but was not completely abolished even at 0°C. It was not inhibited by a variety of enzyme poisons nor by alterations in the ionic composition of the incubation medium, including complete absence of calcium and magnesium. Passive sensitization was inhibited by the presence of non-antibody gamma-globulin in the incubation mixture. The main effect of gamma-globulin was a reduction of the rate of sensitization. However, established sensitization was found to be reversed. That competition for a common binding site was involved was suggested by the interesting finding that although inhibition could be achieved by guinea pig, rabbit and human serum and by purified human gamma-globulin, purified bovine gamma-globulin was inactive. Those results suggested the following conclusion. The reaction is non-enzymatic with a high energy of activation required in the adsorption of antibody gamma-globulin and the desorption of non-antibody gamma-globulin. Antibody and non-antibody gamma-globulins compete for specific cellular attachments. However, a simple reaction like this 'would not account for reversal of established sensitization since the rate of desorption of antibody from its receptors would not be expected to depend on this concentration of reactants in solution'. However, if desorption depended on the concentration of bound receptor, rapid desorption could be achieved. Therefore, they proposed that each gamma-globulin molecule could be attached to a number of receptors. A series of consecutive reactions must be considered:

$$A + R = AR,$$

$$AR + R = ARR,$$

$$ARR + R = ARRR \ldots,$$

where 'A' is the concentration of antibody, 'R' that of free receptors, and 'AR, ARR, etc.' those of antibody bound to one, two or more receptors. Only a small fraction (less than 1%) of receptors needs to be occupied by antibody gamma-globulins for full sensitization. Schild published several review articles on histamine and anaphylaxis (57, 72, 88, 90, 102 and 113).

I summed up Schild's work on histamine a few years ago, 'As can be seen, in over 30 years, Schild was involved in tackling one aspect or another of the histamine affair. His work had profound effects, both theoretical and practical, on our appreciation of the biology of histamine. We doubt if anyone could study any aspect of histamine's role today without being influenced by Schild's ideas'.

2. Autosensitization and delayed hypersensitivity

Going from the pharmacology of histamine to the analysis of anaphylaxis generated for Schild an interest in immunology and its pharmacology which lasted throughout his life. The relationship between acute and delayed hypersensitivity reactions (epitomized by the tuberculin reaction) particularly fascinated him. He investigated two problems: the acute and delayed reactions to autosensitization produced by sperm or testis extracts and the mechanism by which the delayed vascular effects are produced in the tuberculin reaction.

Autosensitization

The original observation was made by Metalnikoff in 1900. He showed that the serum of guinea pigs, which had been injected with their own sperm, developed 'spermatotoxins' which immobilized their sperm. Nearly 50 years later several research groups showed that guinea pigs injected with homologous sperm, or with testis and adjuvant, developed anaphylactic reactions plus testicular atrophy and aspermatogenesis. The presence of circulating antibodies capable of inducing passive cutaneous anaphylaxis had been clearly established. The prevailing opinion was that, in addition to the clear evidence of anaphylaxis, the testicular lesions represented delayed hypersensitivity presumably due to cell-bound antibody.

Schild and his colleagues (76, 85) confirmed the induction of acute hypersensitivity by showing that antigen released histamine from sensitized guinea-pig lungs in vitro, by the occurrence of Schultz-Dale reactions and by the generation of passive sensitization in vitro as shown by histamine release. Intradermal injections of extracts of sperm or testis into sensitized guinea pigs produced strong, ultimately indurating, skin reactions which showed components of both immediate and delayed reactions. In spite of the fact that the delayed reactions were the most pronounced, they were unable to transfer hypersensitivity to naive animals by intradermal injection from sensitized animals. However, they returned to this problem soon afterwards and were able to show that when lymph node cells from sensitized animals were given parenterally to normal animals these animals also developed delayed skin hypersensitivity, thus proving that cell-bound antibody is also involved in sperm-induced autosensitization reactions.

Vascular effects in delayed hypersensitivity

When antigen derived from tubercule bacilli is injected into the skin of patients infected with that organism, they develop a non-acute, local, pathognomonic reaction between 12–24h later involving local vasodilation and oedema, a reaction usually described as 'induration'. Histamine and other substances had been identified as mediators of the acute hypersensitivity reaction; a major question in the later 1950s and 1960s was the nature of the mediators of these delayed vascular reactions. Interpretation of the increases in histamine content and production in tuberculin-reaction lesions (110) was obfuscated by the finding that when histamine production was inhibited, the delayed hypersensitivity lesions lasted longer. Was histamine protective in these delayed lesions?

Characteristically, Schild took a direct and practical approach to the problem. He and his colleagues made aqueous extracts of lymph nodes from normal and

tuberculin-hypersensitive guinea-pigs (92, 96, 109, 115). When the extract was injected intradermally into rats, mice, rabbits or guinea pigs increased vascular permeability at the site was shown by the accumulation of a blue dye given intravenously. They showed by parallel quantitative assays that the permeability effect was not due to histamine, 5-hydroxytryptamine, bradykinin, substance P, kallikrein or globulin permeability factors. The unknown substance (or substances) was referred to as the Lymph Node Cell Permeability Factor or LPF. LPF was present in equal amounts in lymph nodes from normal or hypersensitive guinea pigs. They argued that the enhanced activity in sensitized animals was due to the 10- to 20-fold increase in mononuclear cells in lymph nodes which occur during sensitization and to the arrival of vast numbers of these cells at the injection site.

The activity of LPF was shown not to be due to histamine release. This contrasted with an extract which they made from spleen (132, 142, 150, 151). The permeability increasing effects of spleen extract was clearly shown to be due to histamine release.

3. Development of bioassays

The discovery of insulin in 1921 posed a problem for pharmacologists. There was no chemical method for estimating insulin concentrations in tissue extracts. How, therefore, could these extracts be standardized so as to be sufficiently reliable for clinical use? The initial attempts at biological standardization used biological end points, usually of an all-or-nothing nature, such as death. Estimation of 'animal units' soon proved to be hopelessly unreliable. The individual variation between animals was far too large. For many years, the major emphasis in the development of biological standardization (bioassay) dealt with the realization that these methods had to be comparative, with reference to a standard. The standard had to be stable and be internationally recognized and accepted. Biological standardization was developed for many substances including posterior pituitary extract (as it was then called) and digitalis. (30).

The early assays, so-called 'analytical dilution' assays were done using fixed end-point, quantal, designs. Trevan (1926) showed that there was a sigmoid relationship between dose and cumulated end points. These were interpreted as integrated normal (Gaussian) curves. The experimental designs tried to make comparisons between standard and test preparations over the middle, approximately linear, region of these curves. However, as the variances were not uniform over these ranges various mathematical procedures had to be introduced to 'weight' the data.

Alongside the development of biological standardization for therapeutic purposes, a new need was developing which had to deal with quantitative dose–response relationships. Pharmacologists wanted to measure tissue concentrations of the newly discovered neurotransmitters and autocoids, such as acetylcholine and histamine, which, at that time, defied chemical measurement. Physiologists wanted to relate these concentrations to functional change, neurotransmission, vasodilation, and 'so on and so forth' (as Schild would say). In the 1930s, the standard experimental design for bioassay involving quantitative responses, was the so-called 'matching assay'. The plan of the assay was that standard and unknown samples were compared by trial and error, by bracketing standard and test using guessed dilutions, until they seemed to produce equal effects. An important

advantage of this null method was that no assumptions about the quantitative relations between dose and effect were needed. This was the experimental design that Schild tried to use in his early collaboration with Gaddum to investigate the reactive hyperaemia (increase in blood flow) following muscular exercise. Was it due to the release of histamine?

Schild has recorded his views on these experiments (157): 'I felt dissatisfied with this type of assay, finding it difficult to convince myself that any variations in histamine content had not been due to chance'. The unsatisfactory features of matching assays were that (i) they were inefficient, using only a small part of the data, (ii) the end-point was subjective and (iii) there was no estimation of experimental error. He reacted to these problems in two ways. He tried to reduce experimental error by developing automatic assay machines and he tried to make the designs of his assays more amenable to statistical analysis.

Schild's interest in mechanical devices showed up from the beginning. At the end of his career he wrote (157) 'My basic interest, curiously, was physics; but I became a pharmacologist by a sort of often-encountered compromise.' This latent interest emerged as he struggled to improve bioassay. First, he tried to improve the accuracy of recording; a pulley system (18) proved to be too cumbersome and it was later replaced by an 'approximately linear and isotonic frontal writing lever'. (20). Then he designed an automatic apparatus for making pharmacological measurements on isolated tissues. In this apparatus the emptying and filling of the organ bath was controlled by using telephone relays to clamp the inlet and outlet tubes and their activity was programmed by a telephone exchange rotary uniselector (21). While the assay benefitted from regular timing, the precision of bath volume changes was probably more important. Some years later (49), with the help of Boura and Mongar, he improved the design of the apparatus and, with the support of the National Research Development Corporation, persuaded Casella (Electronics) Ltd to make it available commercially. However, reliability was a problem and the venture was not a great success.

His other approach to bioassay was entirely intellectual. He argued that if the aim of the assay is to detect a chemical concentration, and if standard and test substances are chemically identical, then, 'it does not matter what species or what reaction is used in the test; the final result is always the same and the best method is that by which an accurate result is produced with the greatest economy of time and labour' (80). Starting from this point, Schild published in 1942, in the *Journal of Physiology*, one of the classic papers in the history of pharmacology, although the actual title, 'A method of conducting a biological assay on a preparation giving repeated graded responses illustrated by the estimation of histamine', was hardly inspiring. His idea was that a valid 'null hypothesis' could be set up and tested so that the accuracy of the result would be estimated from the data of the experiment. A major problem in trying to estimate the error variance in a single preparation is that the tissue is slowly deteriorating throughout the course of the experiment. Schild decided to base his experimental design on 'a simple plan used in field experiments on adjacent plots [Fisher, 1938]'. The reference was to another classic, Fisher's 'Statistical Methods for Research Workers', where the procedure of Analysis of Variance was applied to randomized blocks of space (fields) to meet the needs of agricultural research. Schild applied Fisher's design to a randomized sequence of blocks of time to meet the needs of bioassay. The assay,

illustrated by the contractile effects of histamine on gut muscle, started from the assumption that, over a usable range, the muscle contracts linearly in relation to the log of the dose of histamine. Four doses were used, two of standard (high and low) and, similarly, two of the unknown. The assay used blocks of these four doses suitably randomized in sequence. Thus every group of four consecutive doses contains each of the standard and test doses once. The paper then sets out the analysis of the whole data set using words and descriptions which a non-mathematical pharmacologist could easily understand. The analysis of assays using solutions of known composition showed that the method was quite robust, that is it could tolerate some departures from linearity and parallelism of the dose-response curves without serious errors. The design and analysis of the 2+2 bioassay became a standard in pharmacology. Nearly 25 years later (108) Schild published a paper with D. J. Finney to deal with a recurring problem of randomized block, parallel-line assays, in single preparations. The assay works best when the choice of standard and test 'doses' are nearly equiactive. In practice, the initial choice of doses is often unsatisfactory and leads to an inefficient assay or to the need to repeat the experiment with a new set of doses. Finney and Schild's paper explained how the inherently sequential nature of the assay could be used to advantage by changing the doses from block to block. Needless to say the arithmetic was more complicated!

Bioassays work best under two conditions. First when equilibration, or at least a steady state, is achieved between the reagent to be assayed and the tissue reaction to be monitored. In this respect in vitro assays are more likely to be reliable than in vivo assays. Second, they are most reliable when standard and test substances are chemically identical, when concentration is the only unknown. Where two samples differ in chemical composition the 'assay then ceases to be an analytical method and becomes a comparison of biological activity in which species, end-point and experimental conditions become all-important' (30). This second type of assay 'has been named, not very happily, comparative assay. It deals with the problem of comparing the biological effects of two chemically dissimilar substances, expressing the activity of one in terms of the other. This is bound to be a messy affair, since the dose-response relations usually differ; but its practical importance is great, since comparative bioassays form the indispensable basis of all new drug development' (157). After this classic paper of 1942 (18), Schild over the next 25 years, exploited in many directions the basic principles of bioassay which he had established using the interaction between histamine and the guinea-pig ileum.

Schild and his collaborators published a number of papers using gastric acid secretion in the rat as the measuring instrument. Papers published with M.N. Ghosh (53, 70) were mainly concerned with describing a method, but a method suitable for quantitative bioassay. However, in these papers there were examples of comparative bioassays of cholinomimetic drugs and some descriptive pharmacology of the potentiating effects of histaminase inhibitors. Curiously, in retrospect, there were no descriptions of attempts to block histamine or the cholinomimetics by either antihistamines or anticholinergic drugs. Subsequently, Schild used this preparation to develop bioassays for urogasgrone and gastrin (91, 125, 129). In his paper with Rosenoer (91), Schild reported a satisfactory 2+2 randomized-blocks design using six rats to assay the inhibitory effects of urogastrone. In the analysis of variance

only the regression component was significant, indeed highly significant. He calculated that using this four dose assay required 22 times fewer animals than a standard single dose assay.

A few years later (125), Schild and his collaborators used this preparation to assay gastrin and its synthetic analogue 'pentagastrin'. Using, again, a 2+2 latin square design, the analysis showed that there was no significant deviation from parallelism (essential for a satisfactory bioassay) and no significant effect of dose order; however significant variations in sensitivity between the four rats used were found. Clearly Schild's faith in the value of statistical analysis of appropriate experimental designs was becoming established.

In his retrospective analysis (157) and with tongue in cheek, Schild wrote: 'Bioassay is an English disease; I shouldn't think it is taken seriously anywhere else. In Britain, it has acquired the connotation of "pharmacological measurement"'. In my view his diffidence (perhaps he had acquired an English disease?) did us no service. Schild's contributions to pharmacological assay gave our subject incalculable advantages. I had the privilege of sitting-in during his seminars on bioassay at University College London between 1973–1975. They turned out to be much more than expected; they were elegant exercises in clear thinking, an activity at which Schild was a master.

4. Quantitative analysis of drug receptors and their antagonists

Schild always enjoyed trying to solve practical problems. He has testified that applied problems are not only stimulating but also provide a unique introduction to fundamental studies. His studies on drug antagonism serve to prove his point. Although he had been interested in trying to design histamine antagonists while he was at Edinburgh, the opportunity to do something about that didn't arise until 1944. Roche Products had agreed to give an annual grant to University College London provided Schild would act as a consultant. Collaboration with his old friend Franz Bergal was the outcome. They decided to work on antagonists to histamine on bronchial muscle because of the potential importance to the treatment of asthma. As ever, Schild went straight to the heart of the problem. How can drug antagonism be measured quantitatively? He was aware of the Langmuir–Gaddum approach which aimed to give drug antagonism a theoretical framework. The approach was based on the concept that drugs interact, on the basis of mass action law, with specific receptors in tissues. Nevertheless, Schild's initial studies were entirely empirical. At the time he was still strongly influenced by Dale's view, namely that the concept of pharmacological receptors was a redundant idea. Gradually, however, he became converted and, because of his considerable mathematical skills, he subsequently became a formidable proponent of 'receptor theory'.

Schild introduced the pA notation as a new measure of drug antagonism (23). The idea was based on a suggestion by Clark and Raventos (1937) that the activity of drug antagonisms could be estimated by 'the concentration which altered by a selected proportion, e.g. 10-fold, the concentration of an active drug needed to produce a selected effect'. Schild defined pA_x 'as the negative logarithm to the base 10 of the molar concentration of an antagonistic drug which will reduce the effect of a multiple dose (x) of an active drug to that of a single dose'. The pA notation is now firmly embedded in the language of Pharmacology. However, Schild's important contribution was not the invention

of the notation but the experimental exploration and interpretation of its meaning and uses.

Schild analysed his pA measurements using the experimental designs and statistical analysis previously developed for the bioassay of histamine (18). He showed that pA values for antihistamines were independent of the agonist concentrations used for the measurements but were dependent on the time of exposure to the antagonist and the value of the 'dose ratio' determined by antagonist concentration. He used four pA values to characterize an interaction: pA_2 and pA_{10} each measured at 2 minutes and 14 minutes. His only acknowledgement to receptor theory in this paper was a footnote about the difference between pA_2 and pA_{10}. Gaddum's mass action equation for competitive antagonism involving a first order reaction requires a 9× increase in antagonist concentration to achieve the 5× increase in agonist concentration ie $pA_2 - pA_{10} = 0.95$ (= log 9) for a simple competitive interaction. Schild established pA as a statistical constant applicable to a given tissue, antagonist and active drug combination. In such a given system pA values were found to vary by about 0.4 to 0.5 log units. Nevertheless, on the vital matter of whether his results would be reproduced in other laboratories he was not very confident.

Schild's most important use of the pA concept was to introduce a wholly new idea into Pharmacology, the idea of a rational basis for the classification of drugs (24). The very idea that different chemical substances might share a common action, might constitute a specific drug class, was novel. Put another way, the question was whether antagonists, such as the antihistamines, which could easily distinguish histamine and acetylcholine, would be able to distinguish substances which were chemically closely related. Schild again used a randomized blocks design to tackle this question. He found that the antihistamine Benadryl reduced the effects of histamine to the same extent as the unknown substance with histamine-like properties which is released by curare. He then measured the antagonism by three different antihistamines of both histamine and its *N*-methyl derivative; none of them were able to discriminate between the two agonists. He concluded that histamine and *N*-methyl histamine must belong to the same pharmacological class. Schild pointed out that a descriptive classification had been used before in Pharmacology, notably the musculotropic and neurotropic action of acetylcholine-like substances based on the qualitative reaction to atropine. Schild's innovation was to use both quantitative and convergent criteria. He saw the need to use more than one antagonist pA measurement to define the class. His proposed method of classification had the merit of being sharply defined.

Schild (28) was drawn into a discussion of the relation between his empirical pA measurements and receptor theory by Guarino and Bovet's paper (1949). pA was defined as a null method which would be independent of the concentration of active drug used. This condition can only hold when the dose-response curves are 'parallel' when using a logarithmic dose scale. Guarino and Bovet studied the interaction between Flaxedil, a neuromuscular blocking drug, and acetylcholine; Flaxedil steepened the dose-response curves. Therefore the estimated pA would now decrease with increasing concentrations of stimulant drug. They developed a new formula to account for active-drug dependent pA measurements.

The background to this problem was as follows. Gaddum (1937) had produced the first

model of drug antagonism based on the idea of drug and antagonist molecules competing for free receptors on the cell surface according to a simple mass action law. At equilibrium, rates of association and dissociation between drugs and receptors were assumed to be equal. On these assumptions Gaddum derived the formula

$$\frac{y}{100-y} = K_1A = \frac{K_1xA}{K_2B+1}\ , \tag{1}$$

where y = fraction of receptors occupied by the active drug, A and K_1 are the concentration of active drug and its affinity constant and B and K_2 are the concentration of antagonist and its affinity constant; x is the dose ratio, the multiple by which A has to be increased in the presence of B to produce equal effects. Therefore

$$K_2B = x - 1. \tag{2}$$

As K_1 is effectively increased by the factor $(K_2B + 1)$, then the log dose–response curves would be moved in parallel, the necessary condition for the pA definition. In this model B behaves as though it 'dilutes' the receptors. In contrast, Guarino and Bovet proposed that B dilutes the concentration of A so that:

$$K_2B = x(x-1)\,K_1A\ . \tag{3}$$

Schild concluded that their 'assumptions do not appear to be based on mass action competition for receptors in the sense that drug or antagonist occupy a number of receptors for a finite time to the exclusion of each other'. For completeness, in the paper, Schild also considered the case where the antagonist flattens the active drug's dose-response curve. In many cases the decrease in slope is associated with a decrease in maximum as though a constant number of receptors were being put out of action to make this insurmountable antagonism due to non-competitive activity. Schild considered non-competitive antagonism in more detail later on (50, 62, 75).

In his most-cited paper (75), Schild set out his complete thinking on the quantitative uses of drug antagonists. The paper begins: 'Although drug receptors have not *so far* (my italics) been identified by physical or chemical methods, they can be identified pharmacologically by means of antagonists. If two agonists act on the same receptors they can be expected to be antagonized by the same antagonist, and, if the antagonism is competitive, they can be expected to be antagonized by the same concentration of antagonist and to produce with it the same pA_x or dose ratio; pA_x values can thus be used to identify agonists which act on the same receptors. They can also be used to identify receptors in different tissues since tissues with similar receptors would be expected to give the same pA_x with antagonists. Lastly, pA_x measurements can be used to distinguish between competitive and non-competitive antagonists'.

As a professed practical man, Schild seemed to be most comfortable with pA_x as a utility, as an empirical statistical constant. I cannot find in his writings any point at which he asks . . . but what underlies this constancy?. Certainly at some point in his slow conversion from empiricist to theorist, he saw that his empirical $pA_x = \log B$ could be easily inserted into Gaddum's equation for competitive antagonism if Gaddum's equation was simplified and transformed logarithmically.

Thus, Gaddum:

$$\frac{y}{100-y} = K_1A = \frac{K_1xA}{K_2b^n+1} \quad (1)$$

eliminating K_1A and taking logarithms

$$\log(x-1) = n\log B + \log K_2 = \log K_2 - n\,pA_x \quad (4)$$

where, by definition $pA_x = -\log B$.

Thus a plot of log $(x-1)$ against pA_x gives a straight line with slope $(-n)$. The line intersects the pA_x axis at a point corresponding to pA_2 ie log $(x-1)$ = log $(2-1) = 0$. When $n=1$ log $K_2 = pA_2$ and $pA_2 - pA_{10} = 0.95$. Here, then, were two simple tests for competitive antagonism but in Schild's opinion 'it is better to employ a wider range of concentrations of antagonism and test for competitive antagonism by means of equation (4). In fact it was the graphical presentation of this kind of data, leading by linear extrapolation to the intercept on the log B axis where $-\log B = pA_2$, that has become famously referred to as the 'Schild Plot'.

Schild was well aware that underlying Gaddum's model of antagonism was an implied model of agonism. 'In applying the (mass action equation) it is necessary to postulate some relation between receptor activation and response'. However, while he pointed out that a linear function between the number of activated receptors and the tissue response was sometimes assumed, he preferred the more limited assumption that 'equal effects in the absence and presence of antagonist involve equal numbers of receptors'. This limited assumption, he stressed, underlay the use of pA_x and dose-ratio in testing for competitive and non-competitive antagonism. In spite of this, he realized that his pA_x method required that dose-ratios must be independent of the level of response, that is, when doses are expressed logarithmically, the dose-response curves have to be 'parallel'. Total escape from some 'model' of agonism was not possible. Perhaps this explains why he went to such lengths in this paper to prove algebraically (i) that whether the antagonist had singular or plural affinity constants for the receptors the dose-response curves would be displaced in parallel and (ii) that the converse, namely that parallel log dose/effect curves can generally be fitted by an equation of competitive antagonism, was also true. This problem obviously fascinated him, perhaps he wasn't satisfied by his earlier proof, because he returned to it, many years later (118), to use differential calculus to prove that competitive drug antagonism would indeed produce parallel shifts of the dose-response curves.

In contrast to this, Schild had found that the interaction between cinchonidine and acetylcholine on the guinea-pig ileum produced a family of curves in which, although the locations were displaced to the right, the maxima were progressively reduced. Descriptively, the antagonism was insurmountable. In an earlier note (50) he had suggested that 'if it is assumed that the active drug and the antagonist combine reversibly with different sites and that a receptor blocked by antagonist cannot be activated then, at equilibrium, y the proportion of occupied receptors will be reduced by the antagonist K_2B+1, ie,

$$y = \frac{K_1 A}{K_1 A + 1} \cdot \frac{1}{K_2 B + 1} \quad (5)$$

He returned to this problem in the 1957 paper (75) showing that when x_1 and x_2 are the dose ratios corresponding to antagonist concentration B_1 and B_2 then:

$$K'_2 = \frac{x_1 x_2 (B_2 - B_1) + x_1 B_1 - x_2 B_2}{B_1 B_2 (x_2 - x_1)} \quad (6)$$

He contrasted this with his empirical definition of pA_h, namely 'the negative logarithm of the molar concentration of antagonist which reduces the maximum to one half'. In the special case, and only then, when 'antagonism is noncompetitive and the response is directly proportional to the fraction of receptors activated,

$$pA_h = \log K'_2 \quad (7)$$

When he applied this analysis to the cinchonidine-acetylcholine interaction, he found that the log K'_2 values calculated from equation 6 were much more variable and significantly different from the measured pA_h values. He concluded that 'it was doubtful that a true noncompetitive antagonism can be assumed'.

Experimentally, the important parts of the 1959 paper (75) were the data showing that neither mepyramine nor diphenhydramine, classical histamine antagonists, could distinguish, on the basis of pA_x values, between histamine and 2-pyridylethylamine; that the activity ratios of these two active drugs were not altered, as judged by 2+2 assays, by diphenhydramine; that assays based on injections of histamine into the guinea pig, isolated air-perfused lung preparation, where the equilibrating concentrations of histamine or acetylcholine were not knowable, nevertheless gave satisfactory (ie not different) pA_2 estimates for mepyramine, diphenhydramine, pethidine and atropine.

Finally, Arunlakshana and Schild re-examined the interaction between acetylcholine and atropine on the guinea-pig ileum. 'Previous estimates of pA_2–pA_{10} for acetylcholine-atropine gave lower estimates than the theoretical values of 0.95 to be expected for simple competitive antagonism at equilibrium'. Previously values of 0.56 had been found by Schild and 0.73 by Marshall. In designing new experiments, the authors considered two sources of error: possible failure to attain equilibrium and paradoxical potentiating effects of atropine at low concentrations. Therefore, they used a wide range of atropine concentrations in the presence of hexamethonium and in combination with 2+2 dose–response curves in preparations stabilized by over 100 doses of acetylcholine. The log dose–effect curves were displaced in parallel and the slope of the Schild plot slope was 1.04.

In the discussion to this important paper, the authors referred to three problems. First, the use of pA_x for the classification of active drugs required convergent and quantitative criteria. There must be several agonists which cannot be distinguished by pA_x measurements made with more than one antagonist. This would be the consequence of both active drugs and antagonists acting at a single class of receptors. The class of active drugs therefore presupposes a class of receptors. The classification of receptors was also established by the statistical homogeneity of pA_x measurements. In this case pA_x values must be independent of the agonist potency and of the tissue used for the measurement. Thus atropine gave similar

pA_x values in frog heart, chick amnion and mammalian intestine. The frog rectus muscle was an exception, atropine having a pA_{10} value 4 log units smaller than in the other tissues which 'presumably has different, nicotinic, receptors. The finding that different tissues have receptors with similar affinities for antagonists is interesting since it gives support to the notion that receptors are definite chemical entities'.

Finally, in this paper (75), the authors dealt with criticisms of the theory of competitive antagonism raised by the interaction between atropine and acetylcholine (a problem raised originally by Clark in 1926). Clark pointed out that the explanation for the ability of acetylcholine to produce immediate large effects in fully atropinized tissues in terms of simple competition was compromised by the very slow rate at which atropine can be washed out of the tissue. Arunlakshana and Schild concluded that 'if the antagonism is to be competitive it is necessary to postulate either a diffusion barrier for atropine – as suggested by Furchgott (1955) – or a very small fraction of receptors occupied by acetylcholine as suggested by Stephenson (1956) or some combination of the two . . . ' They were gloomy about the possibility of discovering reversible noncompetitive antagonists obeying the mass law quantitatively to determine directly the fraction of receptors occupied. The problem is still with us today.

The 1959 paper has become a classic contribution to Pharmacology. Yet, for ten years the paper was cited only a few times per year until 1970, reached 50 per year in 1975 and only reached 100 per year in 1982. Pharmacologists were slow to appreciate the fundamental importance of this paper.

5. Functional analysis of drug–receptor interactions

Throughout his life Schild used the principles of bioassay and the models derived from drug-receptor theory to try to analyse a wide-ranging agenda of tissue responses to drugs, neurotransmitters and other hormones. He concentrated his efforts on four topics: simplification of tissue responses by eliminating the involvement of nerve cells and fibres; simplification of tissue responses by eliminating changes in membrane potentials; analysis of tissue responses to —S—S— (disulphide bridge) containing polypeptide hormones; and analysis, from the point of view of receptor classification, of tissue responses to histamine.

Elimination of neural structures from excitable tissues

When pharmacologists try to analyse the effects of drugs on visceral tissues, they record changes in the effector cells, which most commonly secrete or contract; these changes may be due to a direct action of the drugs on the effector cells or, indirectly, due to an action on neural elements in the tissue which impinge on the effector cells or to elements of both. Schild used three tactics to try to simplify this problem; he tried anatomical separation, he tried chronic denervation and he studied embryonic, nerve-free, tissues.

Magnus (1904) was the first to show that 'when the longitudinal muscle of the cat's small intestine is stripped off from the underlying circular coat, the ganglion cells of the myenteric plexus of Auerbach adhere to the longitudinal layer and the circular muscle can be freed from ganglia' (44). However, in the 40 odd years since then, a whole series of conflicting pharmacological results had been reported. Part of the problem, as Evans and Schild (44)

found out, was that mechanically separated tissues were much more variable in their responses to drugs than were intact tissues. They studied five different types of preparations – longitudinal muscle plus myenteric ganglia, longitudinal muscle strips without myenteric ganglia, circular muscle strips without myenteric or submucous ganglia, circular muscle strips without submucous ganglia, and circular muscle strips with both myenteric and submucous ganglia intact. Sadly, all this surgical finesse did not generate pharmacological illumination. Overall, they could only conclude that the potentiating actions of eserine were dependent on intact ganglia cells, that nicotine had both excitatory and inhibitory actions independently of ganglion cells and that barium ions acted in an unknown way to potentiate the effects of nicotine and acetylcholine.

A possible source of confusion was that plexus-free muscle strips still contained viable nerve fibres. So they tried to prepare chronically denervated intestinal muscle but their experiments were inconclusive.

Finally, they turned to the chick amnion. The amnion contains smooth muscle cells but is supposed to be free of nerve fibres and ganglion cells. Although the amnion was known to respond to a wide variety of stimulant and inhibitory drugs, Evans and Schild (60) were surprised to find that the preparation also responded to both electrical stimulation and stretch. These responses were unaffected by morphine and atropine and they concluded that the physical stimulation was depolarizing the muscle cells directly. Subsequently (67) they tested this hypothesis by depolarizing the cells chemically, by replacing Na^+ with K^+ ions in the bathing fluid. The effects of electrical stimulation were greatly reduced but, to their surprise, the acetylcholine responses were unaffected. For Schild, this finding opened up a rich new vein of research.

Studies on depolarized muscle fibres

The acetylcholine-depolarization phenomenon produced a flurry of activity which lasted nearly eight years. Schild (68, 75) found that many types of visceral muscle could be completely depolarized when suspended in a medium in which K^+ ions replaced Na^+ ions. The tissues responded initially by immediate contraction but the tension rapidly disappeared. Direct intracellular recording showed that membrane potentials were reduced to zero so that the effects of electrical stimulation were essentially abolished. Nevertheless, all tissues would still contract submaximally to agents such as acetylcholine, histamine, 5-hydroxytryptamine, noradrenaline and oxytocin. Antagonists of acetylcholine (atropine), histamine (mepyramine) and noradrenaline (piperoxan) gave the same pA_2 values in these depolarized tissues compared to normal tissues. Addition of Ca^{2+} ions contracted the preparations and Ca^{2+} was necessary to sustain the drug-induced contractions (81, 82, 89). Calcium-induced contractions of the rat uterus could be relaxed by adrenaline (84, 97) or isoprenaline (105, 106, 111, 112) and the relaxations could be antagonized by dichloroisoprenaline. These actions of Ca^{2+} could be antagonized by Mg^{2+} ions. Schild considered four hypotheses (89) involving combinations of actions at either one or two receptors. He elegantly modelled each of them and established predictions for the ratio of responses corresponding to ratios of $[Mg^{2+}]$. Unfortunately the range of usable concentrations of Mg^{2+} turned out to be too small to allow discrimination between the

hypotheses.

Overall, Schild came to the conclusion, based on the work of Jenkinson and Morton (1965), that the agents acted by producing permeability changes to Ca^{2+} and K^+ and that isoprenaline, in addition to permeability changes, sequestered Ca^{2+} intracellulary, possibly in the sarcoplasmic reticulum.

Metal receptors – tissue responses to —S—S— polypeptides:

In the decade between 1960–70, Schild published a number of reports, papers and reviews on oxytocin and vasopressin (93, 95, 101, 104, 117, 123, 124, 140). Both of these peptides contain a disulphide bond. Schild's studies were based firstly on a report by van Dyke *et al.* (1942) that thioglycollate could chemically inactivate these peptides and secondly on a report by van Dyke and Hastings (1928) that magnesium ions potentiated the effects of oxytocin on rat uterus.

After a series of preliminary reports (93, 95, 101), Martin and Schild (104) published their findings on the antagonism of disulphide polypeptides by thiols. They reported that thioglycollate reversibly antagonized the uterine effects of oxytocin. They showed that the rate of chemical inactivation of oxytocin was too slow to account for the antagonism. The antagonism appeared to be exerted at the receptor level because the action was highly selective (bradykinin and acetylcholine were unaffected) and still occurred in potassium-depolarized preparations. Quantitatively, the antagonism was not compatible with a competitive antagonism of a monomolecular interaction (the Schild plot slope was very steep). Other thiols were also antagonistic but not disulphides, thioethers or non-thiol reducing agents. Of particular interest was the observation that thioglycerol was also an antagonist even though it was a very weak chemical inactivator of oxytocin. The antagonism could be seen in other tissue preparations but not in vivo.

Schild advanced several hypotheses, based on the disulphide structures of the hormones, to account for the antagonism by thiols. Subsequently (124), however, Schild reported that thioglycerol did not antagonize the uterine effects of an oxytocin analogue which lacked a disulphide bridge and concluded that inactivation of oxytocin receptors by thiols could not be the explanation.

Schild then turned his attention to the effects of metals on —S—S— polypeptide receptors (117, 123, 140). The potentiating actions of certain metals, particularly magnesium, manganese and cobalt, had been studied extensively but there were no conclusions about their site of action. Schild chose to use rat uterus (probably his favourite assay) but chemically depolarized so that consistent, graded, responses and absence of conducted impulses would simplify analysis. He found that metals of the first transition series, particularly cobalt, manganese and nickel, were more active at potentiating agonists than the alkaline earth metals including magnesium. Agonists with weak oxytocic activity, such as lys- or arg- vasopressin were much more strongly potentiated than oxytocin. The potentiation was specific for —S—S— polypeptides and the action occurred rapidly and was readily reversible. Schild showed how a set of mass-law equations could be formulated based on the assumption that the metal ions can combine reversibly with —S—S— polypeptide receptors and that the polypeptides could combine with free or with

metal-bound receptors. He assumed, to begin with, that the hypothetical binary hormone–receptor complex would have the same 'efficacy' as the ternary complex of hormone–metal–receptors. However, the resulting model showed that increasing metal concentrations would produce potentiation or inhibition depending on whether the hormone had a greater affinity for metal-bound than for metal-free receptors. The model also predicted that ternary complex formation with metals would produce parallel displacement of the hormone dose-response curves. Finally, the model predicted that in comparing the potency of two metals, the metal dose-response curves would be parallel if differences in receptor affinity were involved but non-parallel if differences in the hormone's affinity for the metal complex were involved. In fact, the metals behaved as if potency differences were due to receptor affinity differences but that the potentiation was due to greater affinity of the hormone for the metal-receptor complex.

Schild compared his findings to enzyme chemistry. Metaloenzymes, with strongly-bound metals, and metal-enzyme complexes, with dissociable metal ions, were recognized. By analogy, Schild proposed that —S—S— polypeptide receptors should be classified as metal receptors. He also suggested that the metals, though not necessary for hormonal activity, potentiated their action by improving polypeptide alignment at the receptor's active site.

Histamine receptors

In 1960, Trendelenberg published a paper on 'The action of histamine on isolated mammalian atrium'. He was the first to use Schild's pA_x ideas for attempting to classify receptors. He concluded: 'Arunlakshana and Schild (1959) observed that pA values are organ and species-independent, and the authors postulate that receptors are characterized by the pA value. If this is true, then cardiac histamine receptors differ from those found in smooth muscle'. Other tissues were known to exhibit histamine responses which were refractory to blockade by anti-histamines. The best known were rat uterus and gastric acid secretion. However, Trendelenberg was the first to apply Schild's quantitative criteria to the classification of histamine receptors. This paper may have been the stimulus to the work published with A.S.F. Ash in 1966 on 'Receptors mediating some actions of histamine' (107). Arunlakshana and Schild (75) had already shown that pA_2 values for the mepyramine-histamine interaction were approximately the same across several tissues – guinea-pig ileum (9.3), guinea-pig trachea (9.1), guinea-pig bronchi (9.4) and human bronchi (9.3). In the new paper, Ash and Schild found that for guinea-pig ileum, the pA_2 values were not distinguishable among five analogues of histamine. He therefore proposed the notation H_1 for the class of receptors for which mepyramine had pA_2 values between 9.1 and 9.4. Ash and Schild then chose two other histamine responses, gastric acid secretion in rat and cat and inhibition of the carbachol-stimulated rat uterus preparation, all of which were totally refractory to mepyramine. He studied nine compounds which were notional analogues of histamine but none of them were antagonists. However, 2-mercapto histamine turned out to be a partial agonist on acid secretion. This is an interesting observation which I am not aware if it has been re-examined. Seven of the analogues were active drugs on both the uterus and acid secretion and their activity ratios were highly correlated (r=0.95). Nevertheless, Ash and Schild concluded that 'classification of other histamine receptors

must await the discovery of specific antagonists'.

From 1969, Schild was a consultant to Smith, Kline and French Laboratories in their programme to find a selective antagonist of histamine-stimulated acid secretion. He was delighted to see the discovery of burimamide and its congenors as selective antagonists. However, he was particularly delighted to see the way the discovery was based not only on the practical use of his pA method but also on the use of his convergent and quantitative principles to establish a new class of histamine receptors. Following Ash and Schild's use of the H_1 notation, the new class was given the notation H_2–.

6. Clinical pharmacology

Schild's medical training, plus his bias towards the useful and practical aspects of pharmacology, gave him an interest in clinical pharmacology and therapeutics which lasted throughout his life. Some of this enthusiasm emerged when he took over the authorship of A.J. Clark's *Applied Pharmacology*. In collaboration with Andrew Wilson he guided that classical textbook through five editions before relinquishing the task in 1980.

However, this interest achieved a much more practical expression when he started to collaborate with the obstetricians of University College Hospital, London. The first problem they tackled (37) was the response of the human uterus and cervix to drugs in early pregnancy. Observations were made on 24 women whose pregnancy was being terminated in the first or second trimester. A metal catheter carrying three balloons in series (two for the uterus and one for the cervix) was introduced, under general anaesthesia, immediately prior to surgical evacuation. They found that the cervix and body of the human uterus were capable of independent contraction. Oxytocin was found to produce contractions of the body of the uterus which increased between the first and second trimester. The cervical responses were, on the other hand, inconsistent, sometimes contracting or no response or even relaxation. On the contrary, the ergometrine series of drugs had characteristic effects namely stimulation of cervical contractions with or without corresponding stimulation of the body of the uterus. Like oxytocin, vasopressin could also stimulate the body of the uterus. Vasopressin and oxytocin were about equiactive in the first trimester but oxytocin was up to twice as active in the second trimester.

The second problem was a classical exercise in the application of the principles of the analytical dilution assay which Schild had helped to pioneer. The particular problem was to compare the activity of a new, wholly synthetic, version of the hormone oxytocin (known as Syntocinon), with the natural extract known as oxytocin BP. They compared the activities of these two materials before labour in women who were to undergo therapeutic abortion, during labour for women who needed oxytocic infusion to treat uterine inertia, and on the post-partum uterus during the second and third days after delivery. No qualitative or quantitative differences between the two compounds were demonstrated nor were any side effects encountered.

The third problem was that assays of oxytocic drugs on an animal uterus in vitro or in vivo or even on a strip of human uterine muscle in vitro often gave a misleading indication of the potency in clinical use. The practical question was about the relative activity of ergometrine and methyl ergometrine. Specifically, the problem was to carry out a

comparative bioassay in post partum women. The uterus involutes very rapidly after parturition so that only about two or at the most three days were available for the assay. Myerscough and Schild (71) used an external strain guage tocograph to measure uterine contractions. They hoped to reduce variability by making several measurements on the same patient. However, they found that two doses on the same day interacted with each other but the doses on consecutive days were possible. Therefore, they used an incomplete randomized block design for the assay in which three doses of each of the agonists were used but each patient received only two doses on consecutive days. The dose-response curves were found to have highly significant slopes and linearity but without significant non-parallelism. These were necessary and sufficient conditions for a satisfactory bioassay. Ergotamine was estimated to be 1.52 times more active than methylergometrine with 95% confidence limits = 0.98–2.7. A feature of this paper was the very clear and detailed statistical analysis.

Retirement 1973–1984

Schild retired in 1973, keeping a room in the Pharmacology Department. For the next seven years he fulfilled, with characteristic diffidence, charm and wit, the important role of elder statesman in the department. He was always available to disperse wisdom and good humour. He continued to play a role in teaching, particularly in small group seminars. His course unit in bioassay, which I attended, was truly a master class. He continued to be thoughtful about various aspects of Pharmacology. His short paper (150) on 'An ambiguity in receptor theory' was particularly perceptive. Otherwise he contributed several reflective pieces (155, 156, 157, 158, 159) on his life, his work and his colleagues which were much appreciated. In 1979, Schild wrote about himself: 'As I feel old age creeping up, I realize its many handicaps. When it comes to quick thinking, my grandson of eleven beats me. When it comes to working in the lab, I realize that I can't or won't do as many things by myself as I used to and that I must either give up or be dependent on outside assistance. In one respect, however, I feel more free than when I was young. I seem to have lived long enough to be able to change my mind' (153). Schild remained very active for several years and acted as a consultant for Sandoz.

From 1980 to 1984, he spent most of his time in the old French village of Roquebrune with his wife Mireille. However, Schild was deeply concerned by the resurgence of Nazism in France in the early 1980s and this depressed him. About his settlement in England, he wrote: 'After nearly half a century, I am still different, if only because my spoken English hasn't much improved. Yet this limbo position has deep advantages, probably good for both sides, though only feasible in a country with such a deeply ingrained sense of fairness'. His interests outside sciences were European literature, music and the politics of moderation.

Sir Willian Paton, in his citation at the presentation of the Wellcome Gold Medal, said of Schild: ' . . . it's fascinating . . . to see how often he was at the start of things . . . Heinz could always be relied upon to express a tolerant view, to try to allow the scientist, young or old, to say what he wanted to say, even if it was not orthodox, entirely rigorous, or fashionable. I don't think this was just pure tolerance; but that there was also a shrewd realization that what seems odd, unexpected, not 'fitting in', today, can be something

important for tomorrow'.

His family was a constant joy to him. He was survived by his wife and their three daughters: Marion, Renee and Barbara.

Honours and awards

1966	Fellow of the Royal Society
1977	Schmiedeberg Plakette of the German Pharmacological Society
1981	Wellcome Gold Medal of the British Pharmacological Society
1982	Honorary fellow of University College London

Assignments

Chairman of the Board of Studies in Pharmacology; Examiner in the Universities of London, Leeds, Liverpool and the West Indies; Member of the Pharmacopoeia Committee; Visiting Lecturer and Invited Professor in numerous foreign universities; Teaching of pharmacology to undergraduate students since 1937; in charge of the pharmacology section of the B.Sc. in Physiology; Supervision of numerous Ph.D. students. Consultant to Smith, Kline and French Ltd and Sandoz Ltd.

Membership of learned societies

Member of the Physiological, Pharmacological, Biometric, and Immunological Societies, and the Society for Experimental Biology.

Acknowledgments

I am much indebted to Heinz Schild's autobiographical notes, to Philippe Durant for help with an earlier draft, to Professor D. Jenkinson for providing archival material from the Pharmacology Department, University College London, to the Royal Society of Medicine Library for helping to compile Schild's bibliography and to Janice Kennedy for typing this manuscript.

The photograph was taken in 1966 by Walter Bird. It is reproduced here courtesy of Godfrey Argent Studio.

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The complete bibliography appears on the accompanying microfiche. A photocopy is available from the Royal Society library at cost.

SCHILD

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