Bernard B. Brodie

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BERNARD B. BRODIE AND THE RISE OF CHEMICAL PHARMACOLOGY

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INTRODUCTION

It is difficult to describe the emotionally charged atmosphere of intellectual excitement and scientific commitment that pervaded the Laboratory of Chemical Pharmacology (LCP) in the National Heart Institute during the years between 1950 and 1970. It was during this period that the leadership of Bernard B. Brodie, together with his ingenious methodological innovations and dedication to scientific pursuit, inspired the intensely motivated and creative environment of LCP. In the following sections we attempt to characterize this period because we believe that historical documentation of science is not only interesting, but also necessary to understand the genesis of new scientific disciplines prior to our dependence on technology and computers.

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BERNARD B. BRODIE

Any attempt to describe the atmosphere and guiding spirit permeating the LCP would be futile without first understanding the character and unusual intellect of its director. Born in Liverpool, England, on August 7, 1907, Bernard Beryl Brodie was the third of five children. In 1911, his family emigrated to Ottawa, Canada, where his father, Samuel, owned a small clothing store. Samuel Brodie worked hard and provided his family with all the basic necessities, but very few luxuries. Brodie's mother devoted herself to her children and was insistent in her determination that each child receive a sound education.

In school, Brodie did not distinguish himself in either general studies or science. Indeed, he fondly recalls his slow childhood development with a remarkable ability to laugh at himself. His language development was far from precocious; he did not begin to talk until the age of four. Even during adolescence, his record was far from exceptional; a high school chemistry teacher once refused to recommend him for a summer job.

When recounting stories about himself, Brodie related them with tremendous personal charm, magnetism, and a unique sense of humor. His eyes would twinkle in delight with a humorous remark, situation, or paradox that he enjoyed telling, often at his own expense. He enticed his colleagues to follow him and his scientific ideas; few could resist his offer to work in his laboratory. All his collaborators have fond memories of the time they spent with him, despite his exacting demands of time and dedication to science.

In 1926, after a disagreement with the principal, Brodie dropped out of high school and enlisted in the Royal Canadian Signal Corps. The army converted a shy, introspective teenager into a confident young man who learned to be aggressive, to box well, and defend himself. Indeed, he became an expert boxer and won the Canadian Army championship in his weight division. This ambition in boxing was not to win or to become a champion, he claims, but simply to avoid getting hurt. The discipline of achieving excellence at something he did not find truly enjoyable stood him in good stead in later years when he needed to master difficult subjects in college and graduate school.

With money won playing poker in the army, Brodie enrolled at McGill University. During his early college years, he found little incentive to work hard or make high grades, even in science courses. His interests had not yet been stimulated and he recounts falling asleep in chemistry class. However, a chance experience during his fourth year stimulated his interests and markedly changed his motivation.

His chemistry professor, W. H. Hatcher, needed assistance with an experiment requiring long periods of careful monitoring. Brodie agreed to help, and

stayed up night after night to obtain data for Hatcher. During this time, Brodie fantasized about the life and questions of science—wondering how ideas were generated for such experiments, frequently envisioning himself as a scientist. Brodie loved this work and was, at last, motivated to work, learn, and understand. His grades soon improved from Cs to As. With help from his chemistry professor, his first paper was published in 1931 when Brodie was only 23 years old (1).

After graduating from McGill in 1931, Brodie enrolled in an organic chemistry program at New York University under R. R. Renshaw. He earned his Ph.D. in organic chemistry in 1935 from NYU, and took a position in the Pharmacology Department with George B. Wallace for $800 per year. In 1937, Brodie published three papers that foretold his future direction: two were published with Wallace in the *Journal of Pharmacology and Experimental Therapeutics* on the distribution of administered iodide and thiocyanate in relation to chloride under both normal and pathological conditions (2, 3); and the third with M. M. Friedman in the *Journal of Biological Chemistry* on a new method to measure thiocyanate in tissues (4). Two years later, Brodie published another paper in the *Journal of Biological Chemistry* on a new method to measure bromide in tissues and biological fluids (5). He also developed assays for calcium and magnesium to determine the effects of these cations on the sleep cycle of dogs.

These papers symbolized the intellectual and experimental pattern that recurred throughout Brodie's scientific career. First, he developed a methodology to understand drug metabolism, disposition, and response. Second, the methodology served as a precursor to the generalization of fundamental principles and underlying concepts.

The ambience in the NYU Pharmacology Department was irresistible, leading Brodie to commit himself totally to a career in pharmacological research. There were two influential persons at NYU who contributed to his commitment: Wallace, a distinguished pharmacologist who had trained in Europe, and Otto Loewi, the Nobel Laureate. Coincidentally, Loewi escaped from Nazi Germany with the help of Brodie's uncle. Brodie admired Wallace and learned from him the importance of formulating a creative and testable hypothesis in well designed experiments. Brodie also learned about the critical role of reliable and accurate methods of measurement, crediting Wallace with transforming him from the organic chemist he trained to be into a pharmacologist and biologist. At that time, pharmacology was largely a descriptive science, but Brodie was singularly responsible for combining the two disciplines by using his knowledge of organic chemistry to create novel methods of drug measurement.

In 1941, the Goldwater Research Service of NYU was directed by James A. Shannon, a fine, young renal physiologist trained by Homer Smith.

Shannon had an unsurpassed gift for scientific leadership, and exhibited an uncanny ability for selecting outstanding young scientists, organizing them into an effective and productive team, and guiding them along the arduous path of difficult problem-solving. He inspired these young men and gave them freedom to express their creativity.

In 1941, Shannon's mission at Goldwater was specific: to develop more effective and less toxic antimalarial therapy. Allied troops were at great risk because the Japanese invasion had cut the world's supply of quinine. He assembled an outstanding group of young, enthusiastic investiators, including Brodie, John Burns, Julius Axelrod, Sidney Udenfriend, Robert Berliner, Gordon Zubrod, J. Tagert, D. Earle, and Murray Steele, whose job was to coordinate clinical and laboratory studies to improve the therapy of malaria. Clinicians and basic scientists were brought together for the first time to solve a fundamental therapeutic problem. This synergistic approach was novel for the academic world, and resembled the subsequent collaboration of scientists in Los Alamos who were brought together to design the atom bomb.

Atabrine had been synthesized and used as an antimalarial, but its toxicity was considerable and its effectiveness against the disease varied. A new therapy was needed, different from that which adjusted the dosage of atabrine according to clinical response. Shannon knew the distinguished pharmacologist Kenneth Marshall from Johns Hopkins University, and was familiar with his brilliant demonstration that dosages of sulfonamides could be adjusted more effectively through blood sulfonamide concentrations. This work implied that a specific narrow range of plasma sulfonamide concentration was associated with a therapeutic effect, whereas lower blood levels were clinically ineffective and more toxic. Shannon suspected that the same principle might apply to atabrine. Until that time, however, atabrine concentrations in plasma and tissues could not be accurately measured with existing methods because of interference from its metabolites.

By extraction in solvents of different polarity, Brodie and Udenfriend separated atabrine from its main metabolites (6). The assay revealed that the drug localized predominantly in liver and skeletal muscle where it was present in several hundred-fold excess over plasma. However, atabrine had to be present in the plasma to attack the blood-borne malarial parasite. The knowledge of atabrine's peculiar distribution in the body provided the foundation for devising more satisfactory dosing regimens. A very high loading dose of atabrine successfully provided effective antimalarial plasma atabrine concentrations. This loading dose was followed by smaller doses to sustain plasma atabrine concentrations at the necessary and effective antimalarial level. A plan for maximizing the benefits of atabrine had been developed by the spring of 1943, less than two years from the start of the effort, largely due to Brodie's assay (7).

Brodie recalls the Goldwater period not only as one of the most exciting in his life, but also as the real beginning of his scientific career. The personal and professional precepts developed through the Goldwater experience were successfully employed throughout different contexts at LCP: the heady exhilaration and excitement of group efforts urgently committed to solve difficult scientific problems; the need to develop new, sensitive, and reliable methods, such as those that successfully separated parent drugs from their principal metabolites; the identification of drug distribution patterns in body tissues and fluids in order to develop rational dosage regimens; and, perhaps the most important, how to foster productive collaborations among basic scientists.

In 1946, Shannon left Goldwater to become director of the Squibb Institute for Medical Research. Brodie remained at Goldwater and, in January, 1947, published a series of six papers describing the separation of drugs from their principle metabolites. These papers emerged from the atabrine work but transcended it by creating broad, general principles that showed how to accurately measure drugs and their metabolites, uncontaminated by one another (8–13).

The generalizability of these methods allowed their application to the separation of other basic drugs from their metabolites. Brodie himself used these methods to describe the metabolism and disposition of drugs common at that time: acids, as well as bases, including acetanilide, antipyrine, aminopyrine, phenacetin, atropine, phenylbutazone, procaine, procainamide, dicumarol, thiopental, and salicylic acid.

In 1949, Shannon was named scientific director of the National Heart Institute and persuaded many of his Goldwater associates, including Brodie, to join him in Bethesda, Maryland. At that time, NIH had little prestige. Many academic mentors of the young Goldwater scientists counseled against following Shannon to NIH. Those who ignored this advice were Brodie, Udenfriend, Axelrod, Berliner, and Zubrod, among others. With imaginations ignited by the Goldwater experience, they determined to succeed. This determination surpassed all dreams. During the twenty year span between 1950–1970, Brodie's LCP was extraordinarily productive; it opened exciting new vistas in remarkably diverse fields, created better methodology, and developed novel scientific paradigms. James A. Shannon, the man largely responsible for creating NIH as it exists today, stated (14):

> "Starting his career with a Ph.D. in organic chemistry from New York University in the thirties, Dr. Brodie moved into biology through quantitative studies on the physiological handling of halogens. He expanded his views on the interaction of chemicals with complex biological systems incidental to his studies, which provided a quantitative base for the rational development of antimalarials during World War II. He has moved through a series of subspecialties in pharmacology and related physiology in a highly productive fashion

since that time. These included the fields of analgesics, antiarthritic and antiarrhythmic agents, and the exploration of the control of CNS function, to mention a few. His activities have resulted in a profound increase in our understanding of drug action, but more importantly, have modified our views on what could or indeed should be done to clarify the complexities of a broad field of concern common to all of us. But then Dr. Brodie's contributions to medicine, and its scientific base, is reflected less in his direct contributions than in the careers of the many scientists who have been affected by his work."

Science in the LCP

This brief bibliography inadequately describes the dynamic nature of Brodie's insatiable quest to discover fundamental biological truths and mechanisms. This was his driving passion, and the commitment and inspiration he instilled in those who worked with him is what distinguished the LCP from other laboratories. This quest often led to endless sequences of experiments lasting days and weeks, punctuated by long discussions and debates on the meaning and inferences of experimental results. If the experimental conclusions supported the obvious, Brodie insisted on another perspective to aim at the unknown and the novel. He penetrated beneath the superficial and the obvious to the profound, conceptually innovative, and biologically meaningful. Thus, the laboratory traveled with him in an exciting journey beyond the existing limits of knowledge. Future experiments would generate something entirely new, something previously unsuspected. The stimulating challenge of discovery made the researchers in the laboratory more than willing to endure the many associated difficulties and discomforts.

The characteristic of relentless inquiry explains why, for Brodie, there were no simple, straightforward results. Experimental data possessed wider significance, if only one would take the time and trouble to search for the more complete meaning. Results of experiments gave hints and clues of the overall, larger picture: the important underlying biological principle. Brodie's particular gift was to detect and extract information from isolated results, which often concealed the overarching concept and the unifying idea among the data. In other words, his course was never deterred by petty detail, and he maintained that it would be unfortunate and unwise to let a good idea expire on the basis of a poor experiment or incomplete "facts".

Detail annoyed him, as, for example, undue concern about the statistical significance of results. If a scientist had to rely heavily on statistics to discover a basic, organizing pattern, he felt, something was amiss. An overreliance on statistics might encourage the vision of a new biological principle that was, in reality, nonexistent. One did not need statistics when a bona fide phenomenon emerged.

Brodie's approach to the scientific literature was similar: if it was necessary to consult the literature to determine whether an idea was new and worth

pursuing, it probably was not. Any new and worthy experiment would, by definition, not have been conceived and executed previously. Therefore, lengthy literature searches were unnecessary.

While such attitudes may surprise contemporary scientists, they were justified in Brodie's era. He possessed a remarkable, if not unique, intuition and sensitivity for uncovering broad biological principles and for sensing what was biologically significant and what was not. Thus, his contributions were original, innovative, and represented major departures from contemporary dogma. His mind sought connections between diverse facts and multiple experiments, always careful to avoid superfluous detail and unimaginative, limited conclusions. Brodie continually challenged himself to synthesize disparate phenomena for the purposes of conceptual unification, in much the same way as Einstein attempted to synthesize diverse phenomena in physics for the purpose of a general field theory.

To do this, Brodie continuously asked questions, often in rapid fire succession, but always orderly and logical, progressing from the seemingly elementary to the increasingly complex. Questions flowed with animation and vivacity as though they were part of his inner nature and needed immediate release. This process was, of course, the Socratic method of discussion—a series of progressive questions, each designed to test and advance beyond the preceding answers, ideas, and insights to approach the truth.

Brodie loved, and wisely availed himself of, the flourishing intellectual environment at NIH which abounded with experts. In many discussions with the NIH authorities his continuous line of debate and questions, seemingly uninspired at times, led ultimately to new insights and the emergence of experimentally testable hypotheses.

Brodie's urge to pursue new knowledge often occurred in the early morning hours when he would invite colleagues to his house to participate in long Socratic discussions. If an erroneous hypothesis occasionally led them astray, it would self-correct when an experiment revealed the error of a particular idea and a new and better idea would replace it. As unquenchable as was Brodie's appetite for asking questions, his appetite for suggesting, developing, and performing experiments was even keener. Brodie's experimental designs were generally direct, straightforward, logical, and easily reproducible. Excuses for not performing experiments were unacceptable: in his view they were always possible. He was fond of saying that experiments would never be performed if scientists allowed themselves to be talked out of doing them.

It is indeed remarkable how many brilliant, new, unifying concepts Brodie was responsible for discovering in his career. These will be discussed in later sections, but in conclusion, a frequently quoted and misunderstood remark of Brodie's, "Let's take a flier on it", needs to be explained. This remark has

been attributed to Brodie's penchant for gambling and following far-out scientific hunches. His close friends named him after the legendary bartender and gambler, Steve Brodie, who, on a bet, jumped off the Brooklyn Bridge and won by surviving. An amusing anecdote to Bernard Brodie's scientific style perhaps, but it incorrectly conveys the role of chance in his scientific career, a false impression propagated in Robert Kanigel's book *Apprentice to Genius* (15). This book, generally accurate and stimulating, deserves much credit for its insights and for being the first to describe in detail Brodie, LCP, and its rich environment. Kanigel wrote that Brodie's favorite challenge—"Let's take a flier on it"—embodied a style of science characterized by a breathtaking freedom to be wrong. On the contrary, when Brodie suggested an experiment, he was convinced that the effort would not be wasted, but rather would advance in logical, rational, and necessary steps on a path ultimately leading to truth. The intention of a scientific gamble, or flier, was to stimulate, even entice, his colleagues into viewing things from his perspective in order to perform often arduous and tedious work. Brodie's remarkable ability to perceive the big picture and his intuition for the discovery of novel scientific principles made most of his experiments a success. Because an astonishing number of his concepts received experimental confirmation and opened new areas in pharmacology, Brodie, who published approximately 400 scientific papers, is considered by many to be the father of modern biochemical pharmacology and neurochemical pharmacology.

Contributions of LCP to Drug Metabolism and Disposition

The contributions made by Brodie and LCP to drug metabolism and disposition were numerous and fundamental. Although he was the director of the LCP and indisputably the intellectually dominant figure, he acknowledged the critical role played by his close associates. The LCP team was large, its composition continually changing and constantly enriched by new members. Indeed, there were literally hundreds of scientists trained there who left for subsequent scientific positions in other institutions armed with the concepts developed at LCP. They also took with them the inspiration, excitement, and voracious appetite for novel experiments that characterized the spirit of the LCP. They too thirsted for a fresh vision of nature. Many became directors of laboratories and institutes throughout the world and are today considered international leaders in pharmacological research and in the neurosciences: a measure of their success.

We had intended to include in this reminiscence a list of such scientists, but it was too long; it kept growing as we tapped more sources and questioned more colleagues. Such a list, too, would not include those who did not serve an apprenticeship at LCP but were nonetheless profoundly influenced and even transformed by the ideas and principles generated there. A count of guest

scientists from foreign countries who trained at LCP from 1950–1970 yielded 79 names from 29 different countries. Thirteen others received their doctoral degrees in pharmacology from George Washington University for research performed in LCP under Brodie's direction. The scientists there were from different disciplines and countries and provided a rich intellectual environment; without this, the enormous achievements and discoveries that emanated from LCP would have been impossible.

One notable feature of the research performed at LCP in the traditional area of drug metabolism and disposition was the extraordinary array of topics, problems, drugs, and approaches; that is, the staggering diversification of themes. The basic and applied implications and significance of these themes characterized the wide significance of LCP research. Brodie's fundamental contributions to analytical methodology were based on principles of differential extraction of drugs and their main metabolites by solvents of different polarity. These papers had such a profound influence on pharmacology and medicine that it is difficult to overestimate their importance. Present day assays of drugs and their metabolites depend on these principles; new drugs cannot be approved without first obtaining information on metabolism and disposition.

Other applications of these assays include (*a*) studies on drug interactions; (*b*) pharmacogenetics (individual differences in drug elimination due to genetic factors); (*c*) environmental and developmental factors that influence rates of drug clearance; (*d*) diagnosis and management of poisoned and overdosed patients; (*e*) assessment of patient compliance with medication regimens; and (*f*) selection of appropriate routes of drug administration, dose, and dosage intervals. This list exemplifies the principle that the more fundamental and basic a discovery is, such as the idea that drug and metabolite concentrations can be quantified through the use of solvents of different polarity, the broader the application.

Another fundamental discovery made in LCP was that of the mixed function oxidases or drug-metabolizing enzyme system. This system, also designated the cytochrome P-450-mediated monooxygenases, is located in the smooth endoplasmic reticulum in liver cells and other tissues. It requires molecular oxygen as well as NADPH and was first described in 1953 (in abstract form) by LCP's Bert La Du, who showed how it was responsible for the hepatic demethylation of aminopyrine (16). It was probed and described in several additional papers from LCP (17–23).

In 1953, Axelrod was also working in LCP on the same problem and described (in abstract form) the actions of this hepatic enzyme system on amphetamine and ephedrine (24). The significance of the enzyme system has only been fully appreciated in the last few decades with the recognition of its central role in the detoxification, and occasional activation, of many drugs

and foreign organic chemicals. Brodie recognized the biological significance of this discovery when it was made in his laboratory in 1953. It was customary for him to have several different individuals or groups in LCP work on the same problem, if he considered it particularly important. He recalls that his first intimation of the universal aspects of this enzyme system came from the capacity of SKF-525A to inhibit the metabolism of markedly different substrates. The system's broad substrate specificity was not lost on Brodie, and he suspected that a fundamental principle would underlie a system functioning to detoxify such a wide variety of foreign chemicals.

He postulated a grand evolutionary scheme: aquatic creatures did not need such a system for detoxifying foreign chemicals because they could readily eliminate lipid soluble foreign compounds through their gills. But as vertebrates evolved to land-living forms, they required enzymes that catalyzed disposal of lipid soluble alkaloids present in their foods; otherwise such compounds would remain in fat depots indefinitely. Drug-metabolizing enzymes allowed plant alkaloids and other lipid-soluble environmental chemicals to be converted to more polar, and hence, more excretable metabolites. Thus, their activity increased progressively as life evolved from the creatures confined to the sea to amphibians, reptiles, birds, and mammals (25).

His evolutionary hypotheses inspired studies on species, strain, and sex differences in rats, as well as pathways of drug metabolism. Brodie shared this interest with his longtime friend, R. T. Williams, from St. Mary's Hospital, London. Perhaps the best known expression of Brodie's commitment to investigating species differences is the paper by Quinn, Axelrod, & Brodie (26).

A corollary of the research addressing the evolution of drug-metabolizing systems in vertebrates was the investigation of the activity of drug-metabolizing enzymes in the neonatal period. Brodie's ontogenetic studies revealed that the drug-metabolizing enzymes in newborn mammals are greatly reduced or even absent. These observations had profound implications and explained the long-recognized increased sensitivity of newborns to many drugs and other environmental chemicals (27). It also reaffirmed the fundamental biological principle that ontogeny recapitulates phylogeny.

The identification of large differences among normal human subjects in their rates of drug elimination was influenced by the studies on species and ontogenetic variations in drug metabolism, the development of methodology to measure drugs and their metabolites, and the discovery of the drug-metabolizing enzymes. Although individuals often differed by as much as 10-to 20-fold in the rates of drug elimination, repeated measurements on the same individual were highly reproducible. Stimulated by Brodie's probing questions, twin and family studies were conducted using a wide variety of drugs metabolized by this enzyme system. These studies revealed genetic

control of large interindividual differences. In identical twins, metabolic variations in drug elimination vanished, but in fraternal twins differences were largely preserved. As genetic variation disappeared, so too did pharmacokinetic differences. Yet other studies in LCP revealed that environmental factors could play important roles in affecting rates of drug elimination. One of these was exposure to numerous inducing agents, such as phenobarbital. Response to inducing agents was not uniform; rather, it was variable and influenced by genetic factors (28). Clearly, genes and environment dynamically interact to cause interindividual, as well as intraindividual, differences among patients in their response to drugs.

Many studies emanating from LCP using Brodie's methods for measuring drugs and their metabolites involved drug distribution. Fundamental principles about the influence of pH on the passage of drugs across lipid membranes emerged from these studies. Acidic drugs under acidic conditions were shown to be lipid soluble and, hence, crossed membranes most rapidly. Conversely, basic drugs rapidly transversed lipid membranes under alkaline conditions. These fundamental principles had many practical applications in medicine as well as in the pharmaceutical industry in designing products for maximal absorption.

Two of Brodie's major contributions can be cited as examples (29, 30). The first correlated the distributional properties of thiopental with its clinical characteristics. Its rapid onset of action followed from its lipid solubility, derived from its being largely nonionic at plasma pH, and its very high partition coefficient. These factors accounted for its rapid uptake by the brain. Its short duration of action as an anesthetic, lasting approximately five minutes, initially appeared attributable to rapid hepatic metabolism. However, after a series of questions, the redistribution from brain to other tissues as plasma thiopental levels decreased was shown as a critical event. From plasma, thiopental localized in adipose tissue.

The second example concerns the role of plasma protein binding of a drug in determining its clinical properties. It is also illustrated by thiopental: its rapid uptake by the brain occurs in part because it is negligibly bound to plasma proteins. Since this is a negative instance of an important principle, we will illustrate the principle with a positive example: Brodie and his associates showed that the long duration of action of phenylbutazone and dicumarol arises from their extensive and tight binding to plasma protein.

Another fundamental principle emerging from LCP concerns the potential pharmacological interest and clinical utility of drug metabolites in their own right. Biotransformation of phenylbutazone to the pharmacologically active oxyphenylbutazone (Tandearil^{R}) and to another metabolite that ultimately yielded sulfinpyrazone (Anturan^{R}) exemplified this concept (31).

Space limitations prevent a description of all the major contributions by

Brodie and the LCP to the field of drug metabolism. The final example is chronologically the last in which Brodie was the main contributor and, once again, is anecdotal. On a visit to a sheep station while vacationing in Australia, Brodie was told that CCl_4 was used to deworm sheep. Almost all the sheep tolerated CCl_4 well, but an occasional one died of hepatic necrosis. It was known that pretreatment of sheep with phenobarbital greatly increased the liver toxicity of CCl_4. The hepatic drug metabolizing system was not acting to detoxify foreign compounds, but rather to activate them to produce transient metabolites, such as epoxides. Brodie perceived that the highly reactive intermediates could initiate processes leading to tissue necrosis, mutation, carcinogenesis, and teratogenesis through covalent bonding to critical cellular macromolecules (32). Many LCP researchers participated in this landmark work that covered a broad range of toxic metabolites formed from such chemicals as carbon tetrachloride, bromobenzene, and acetaminophen. Key contributors on the research team included Jim Gillette, Jerry Mitchell, David Jollow, Watson Reid, Jack Hinson, and Bill Potter. This work exposed a general principle of broad biological significance; few examples of toxicity from environmental chemicals are excluded by it. The proof involved subtle use of chemicals that act on the drug-metabolizing enzymes, specifically pretreatment with inducers such as phenobarbital that enhance toxicity or with inhibitors such as piperonyl butoxide that reduce toxicity. Again, the practical applications of this fundamental original concept were enormous. In fact, their importance unfolds to this day.

The Serotonin (5HT) and Reserpine Connection

A series of studies conducted in the LCP on the relationship of 5HT and reserpine marked the beginning of the discipline of Neurochemical Pharmacology. These studies pioneered a novel methodological strategy using drugs as tools; they were used to expose neurochemical changes of physiological relevance and then to study the relationship between neurochemical and physiological change. This strategy, developed over three decades ago, is still used repeatedly in several laboratories throughout the world, and was instrumental in uncovering the relationship between 5HT and reserpine. This drug was observed to metabolize rapidly in the body; however, its action persisted while drug plasma and tissue levels were undetectable (33). Researchers in Brodie's laboratory noticed a structural resemblance between reserpine and 5HT and examined the effects of reserpine on the urinary content of 5-hydroxyindole acetic acid (5HIAA) a major 5HT metabolite. It was found to be markedly elevated. In 1955, many laboratories speculated about the role of 5HT in brain function; with the technology to measure it (34), researchers at LCP were able to show that the 5HT content of rat and rabbit brain would become undetectable after an intravenous injection of reserpine (35). After reserpine, brain 5HT became undetectable for longer

than 24 hr; this decrease persisted long after reserpine was virtually absent in tissue extracts. Brodie and his collaborators interpreted this as evidence of a reserpine-induced syndrome related to an inhibition of 5HT storage caused by undetectable amounts of the drug bound to a specific process operative in 5HT storage. From these studies, Brodie and his colleagues suggested that reserpine inactivates a mechanism essential for 5HT storage (36).

Since reserpine elicits a long-lasting symptomatology characterized by immobility, increased muscle tone, blepharospasm, salivation, chromodacryorrhea, hypotension, and other cardiovascular changes, the time course of the symptoms evoked by reserpine was discovered to be related to the depletion of brain 5HT (37). It was suggested that the depletion of brain 5HT was central to explaining the syndrome evoked by reserpine. Later, other laboratories reported that brain norepinephrine and dopamine contents were also depleted by the reserpine action on the monoamine storage process suggested by Brodie (36). This catecholamine depletion helped to explain some of the symptoms of the reserpine syndrome.

Drugs, Neurotransmitters and Function

Several different paths of research were opened by the initially exciting findings on the mechanism of action of reserpine. Following the demonstration in LCP that the long-lasting effects of reserpine were associated with the depletion of 5HT, and despite the evidence that brain catecholamines were depleted, a series of studies led Brodie to establish that the impairment of brain 5HT storage was operative in the sedative action of reserpine (38–41).

The heuristic and novel idea of explaining drug actions via their effects on transmitter function was exploited in the LCP with such drugs as monoamine oxidase (MAO) inhibitors, antidepressants, and hypotensives. As with reserpine, it was necessary to determine whether the central actions of MAO inhibitors were related to the effects on brain 5HT or norepinephrine. The pertinent studies showed a relationship between the characteristically delayed action of some MAO inhibitors to the gradual increase in norepinephrine levels (42). The resulting excitatory action of MAO inhibitors, such as iproniazid, was compared in Brodie's laboratory with the association of reserpine and a new category of drugs, tricyclic antidepressants, which included imipramine (43). Brodie recommended that 5HT rather than norepinephrine may be involved in the antidepressant action of imipramine (44, 45). These studies, combined with the classical drug metabolism approach of LCP, led to the use of desmethylimipramine in depression (an imipramine metabolite, particularly abundant in rats receiving imipramine). The early concept of the anti-5HT mechanism of the action of LSD was consistent with Brodie's demonstration of the importance of 5HT in brain regulatory mechanisms (46).

It is worth remembering that researchers at LCP initiated biochemical studies of the 'adrenergic neuron blockers'—bretylium and guanethidine (47). The demonstrated action of these drugs on the sympathetic nerve terminals was defined at LCP on the basis of their selective depletion of heart norepinephrine stores; bretylium did not change heart NE content, which is descreased by guanethidine (47, 48). Furthermore, Brodie stressed the importance of the physicochemical characteristics of these drugs acting solely at the periphery (48, 49). One important implication of these studies is their clinical application in the treatment of hypertension.

Altogether, these studies led to the development of important concepts of neurochemical pharmacology that included brain storage, uptake, and release of monoamines, and the role of MAO as a regulatory process of neuronal monoamine content. It should also be recognized that this work preceded the histochemical documentation using light and electron microcopy that showed that neuromodulatory monoamines are stored in the synaptic vesicles of specialized neuronal systems and that MAO is located in mitochondria. There are many representative publications illustrating the importance of this work (50–53).

An important aspect of these studies was their emphasis on the relationship between the pharmacodynamic and behavioral effects of drugs acting on the central and peripheral nervous system, particularly as it relates to their actions on monoamine stores. Besides the tranquilizing and sedative action, effects ranging from anticonvulsant to antihypertensive actions were described in detail to depend on their action on the dynamics of neuronal monoamine stores (54, 55). There was, of course, much interest in these studies as they emphasized the functional significance of the changes in turnover rate of peripheral and central monoamine transmitters. There are several illustrative examples of this approach (56, 57).

The interest in the functional aspects of drugs affecting brain monoamine synthesis and storage led Brodie to creatively suggest that 5HT and norepinephrine are regulatory agents of the central sympathetic and parasympathetic systems (58). An even broader view of the function of these amines could be developed along the lines of the physiological concepts earlier proposed by Hess (34). As formulated by Brodie, autonomic, extrapyramidal, motor, and psychic functions are coordinated by two opposing subcortical divisions: ergotropic and trophotropic (59). They are represented particularly in the posterior and anterior hypothalamus, respectively, and in additional diencephalic areas. In fact, the LCP accumulated behavioral, pharmacodynamic, neurochemical, and other evidence to show that free brain 5HT produces actions opposite to those of free norepinephrine, while LSD mimics the effects of the latter and antagonizes the effects of 5HT and its precursor, tryptophan. In other words, free brain 5HT is trophotropic and sedative, while

free brain norepinephrine is ergotropic and excitatory. There is a certain ambiguity in this reasoning, however, because the tranquilizing and sedative drug, reserpine, is a 5HT depleter; but 5HT is a trophotropic agonist. However, Brodie distinguished between depletion on the one hand and the free 5HT, on the other, that resulted from normal or accelerated synthesis of 5HT associated with the inhibition (by reserpine) of the process that stores and protects 5HT from uncontrolled release. As a result, reserpine could cause the unregulated availability of the neuromodulator to its specific receptors. Some studies partially represent these ideas (45, 50, 59). Brodie's demonstration of the functional and behavioral role of central monoamines supported their role as central neuromodulators or neurotransmitters.

Transduction, Modulation, Turnover and Second Messengers in Transmitter Function

In the late 1950s and early 1960s Brodie was somewhat unhappy with the concept of the monotypic chemical signal in synaptic transmission, on account, perhaps, of his awareness of the biochemical heterogeneity of nervous tissue, or of the interplay between 5HT, catecholamines, and acetylcholine, the parasympathetic neurotransmitter, which had been demonstrated at the LCP. This thinking influenced his concept of the synapse (particularly those activated by the sympathetic nerve endings) as a "highly organized molecular unit . . . the neurochemical transducer . . . mediating . . . the change of one kind of energy into another" (54). In this complex functional structure of the 'neurochemical transducer', a number of processes were controlled in the presynaptic terminals, including the kinetics of the neurotransmitter turnover that kept enough transmitter in storage, even in the face of wide changes in the release rates. He also was aware of the crucial role played by the postsynaptic transduction of the synaptic signal into a specific message for the postsynaptic neurons via enzymatic processes that were subsequently indicated to produce specific second messengers activating important metabolic processes (phosphorylation) in the postsynaptic cells. This was the beginning of our current understanding of polytypic- or polytransmitter-synaptic signaling. This process, operative in the regulation of synaptic strength, is changed by the quality of the interaction of synaptic signals and can become somewhat independent from the amount of the primary transmitter that is released. This was a more innovative and dramatic concept than the classical conception of monotypic synaptic signaling of the late 1950s and early 1960s.

The kinetic studies that maintain transmitter steady state in the monoamine stores located in presynaptic endings soon led to the concept of the dynamic state of transmitter storage regulation. By measuring the oscillation of this dynamic state associated with functional changes, drug action in a given

neuronal system could be assessed. This measurement, known as the evaluation of transmitter turnover, became an important procedure in the detection of indirect drug action on a given neuronal system (60). In establishing this concept at the LCP, the necessary methodological and mathematical analyses were formulated for the measurement of transmitter turnover (61, 62). These measurements included the pioneer use of radiolabeled transmitters and their precursors to assess monoamine turnover rates. At this time, it was reasoned that a measurement of transmitter turnover would allow the assessment of the degree of functional participation in a specific brain structure of a given neuronal pathway. Today, the profiles of drug action on the CNS are routinely studied by measuring the turnover of various transmitters in different brain structures, rather than measuring changes in transmitter levels. Furthermore, the methodology originally established for the measurement of the turnover of norepinephrine was adapted to the measurement of the turnover of other transmitters (e.g. 5HT, acetylcholine, and the amino acid GABA).

No less important were the advances made in the area of synaptic modulation by receptor–receptor interaction. The LCP studies that reported how specific ganglionic catecholamine stores regulated cholinergic ganglionic transmission constituted the first demonstrations of both the phenomenon of modulation and the participation of this phenomenon in the regulatory mechanism contributing to the individual diversity of brain function. As stated at the time, the results obtained with reserpine and monamine oxidase inhibitors "support the notion that norepinephrine in the ganglia modulates the action of acetylcholine" (63). It is now known that both pre- and post-synaptic modulation of transmitter receptors participate in the regulation of synaptic strength.

The unique discovery of the changes of synaptic strength through receptor–receptor interaction remained dormant for over 15 years; when revived, however, it guided the study of the mode of action of benzodiazepines. Brodie participated in these studies after he retired from NIH. Indeed, as it turned out, these studies led him to formulate the concept that a homeotypic receptor modulation was involved in explaining the role of GABA receptor modulation to understand the molecular mechanisms whereby benzodiazepines exert their anxiolytic action (64).

Other advances were made at the LCP on the concept of signal transduction at synapses and its involvement in the generation of a second messenger. Important initial studies were carried out on the second messenger role of cyclic nucleotides in the FFA mobilization by sympathetic neurons. If time had permitted, Brodie would undoubtedly have demonstrated the second messenger role of the cyclic nucleotide system in the pharmacodynamic response of 5HT and catecholamines. Many representative publications exemplify this work (65, 66).

The principles discovered at LCP are still extensively applied in many

laboratories as tools to study the mechanism of drug actions on the CNS, and have become standard procedures for determining the neurochemical profiles of drug actions in the central nervous system. In homeotypic receptor regulation the process of allosteric modulation of the primary transmitter recognition site changes the probability of the primary transmitter action. In summary, under Brodie's leadership (63) the LCP has provided the seminal observation that synaptic strength can be modulated by the quality of multiple chemical signals released from nerve terminals and can thereby become relatively independent of the changes in the amount of primary transmitter released.

Two major findings at LCP have directed brain researchers to discover heterotypic and homeotypic regulation of transmitter receptor excitability. In 1961, while studying the transmission of nerve impulses in cervical sympathetic ganglia of cats and rabbits receiving reserpine, it was found that one or two hours after reserpine injection the transsynaptic excitation of nicotinic receptors were facilitated (63). Such a facilitation was possibly mediated by the reserpine-induced depletion of specific stores of ganglionic biogenic amines operative in synaptic strength modulation. Extending this mechanism to the brain, we now recognize many examples where monoamines are not primary transmitters, but exert a modulatory role on the excitability of other transmitter receptors.

Brodie's second major contribution can be seen in the work on GABA receptor homeotypic modulation (64). Through this work, it is now widely accepted that a heterotypic receptor interaction and a homeotypic receptor modulation participate in synaptic transmission variability, which probably is a component for individual brain function variability.

Hypothalamico-Pituitary-Adrenal Axis, Psychotropic Drugs and Homeostasis

Brodie suggested that reserpine stimulates the pituitary-adrenocortical system and causes a hypersecretion of ACTH (37). Ultimately, ACTH depletion ensues. Additional work established the relationship between FFA mobilization, glycolysis, thermocontrol, and catecholamines; in addition, the parasympathetic nervous system was also shown to be a necessary part of the physiological mechanism (65) involved in energy and temperature regulation. Thus, cholinergic agonists such as tremorine or physostigmine evoked the loss of body heat in animals exposed to low environmental temperature and decreased the rate of utilization of energy substrates, while catecholamines exerted the opposite effect. The effects of catecholamines, as well as of cold, on the mobilization of FFAs and glucose depended on the integrity of the hypothalamico-pituitary-adrenal axis.

Peripheral way-stations were involved in these effects as well, and some of

these phenomena depended on the intactness of the sympathetic ganglia and on the activation of the adipose tissue lipase by the sympathetic nervous system (67). Altogether, these studies demonstrated that the autonomic, central, and neurohumoral parameters underlie Claude Bernard's principle of homeostasis (37, 68, 69).

CONCLUSIONS

Brodie established chemical pharmacology and developed many research trends in biochemical and neurochemical pharmacology that are still contemporary points of departure for research on drug action. He was well-loved and respected by his many colleagues, and to this day his work instills enthusiasm, dedication, and intellectual excitement in those who were fortunate enough to work with him. He created many important and distinctive fields of inquiry in our discipline that are functionally alive; his work guides us in our quest to develop new and more effective drugs for the treatment of various pathological states.

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