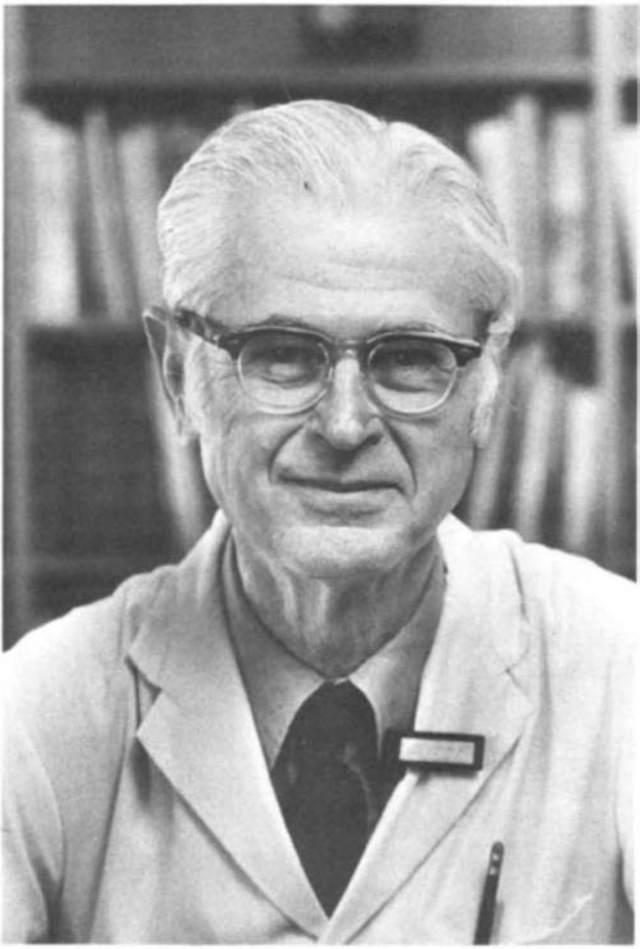

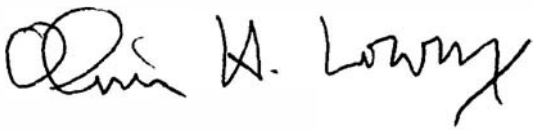

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HOW TO SUCCEED IN RESEARCH WITHOUT BEING A GENIUS

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GROWING UP

Ever since receiving the invitation to write this prefatory chapter, I have been wondering "Why me?" After reviewing the list of previous authors of this chapter, I was even more puzzled until I realized that this may have been a move to show that it is not necessary to be a genius to contribute to science.

I grew up in a very religious family with ancestors on both sides of the American Revolution. Several ancestors were preachers. One exhorted our soldiers in the 1812 War to "fight with the sword of the Lord and of Gideon." Another was John Rankin, a prominent pre–Civil War abolitionist who had a price on his head in Kentucky (which he ignored in his travels). He operated a very successful station on the underground railroad at the Ohio-Kentucky border where he passed 2000 slaves North without a single loss (Harriet Beecher Stowe wrote the part about Eliza crossing the ice from his house on the Ohio River). But as far as I know, none of my ancestors was a scholar or a doctor.

My father was the son of a carpenter who was killed during a barn raising, leaving an impoverished family, held together by a determined mother with a reputation for uncommon sense and a great respect for education. She also had a wholesome level of skepticism expressed by "what *they* say is a lie, and what *they all* say is a lie and a half."

At age 19, my father started teaching in a one-room country school and began a program of self-education. He managed in his early 20s to get a job teaching physics in the Chicago school system, where he introduced the first physics laboratory in the city. He was a master of the Socratic method. Whenever as a child I asked him a "why" question, he would always respond by asking me a series of questions to show me that I, myself, could figure out the answer. His use of this technique, I believe, had an important influence on my eventual attitude toward scientific problems. I recall a specific reinforcement of this attitude as a graduate student: I needed to know certain physical properties of a particular compound. I knew that my thesis advisor would not know the answer—the answer was probably not in the literature—but I could go into the laboratory and in a short time determine the answer. This reinforcement of my father's teaching, and the confidence it gave me, may have been the most important lesson of my graduate training.

My father went on to become a school principal, then a district superintendent, and finally Acting Superintendent of all the Chicago public schools. Not being much of a politician, he never became the permanent Superintendent, but ended his career as superintendent of all the Chicago high schools.

During most of his career, he was continuing his program of self-education. He arranged for an individualized degree program with Northwestern Univer-

sity, which he carried out as a district superintendent by studying on the "Elevated" going from school to school. He would split each textbook into segments that would fit into his pocket. After getting a bachelor's degree this way, he started on a PhD program with a thesis designed to test for innate musical ability among his public schoolchildren. Unfortunately, after years of testing and documentation, his thesis material was accidentally discarded, and he never found time to start over.

All of his children were provided an opportunity (on his teaching salary) to obtain advanced degrees: my sister an M.S. in mathematics; my three brothers, respectively, a law degree, an advanced engineering degree, and a PhD in organic chemistry (this last with postdoctoral training under Willstaetter in Munich).

As the youngest child, I felt I had to live up to much of what my admired siblings accomplished. I never aspired to the law, but conceived of combining chemistry and engineering to emulate my two oldest brothers. Unfortunately, my youngest brother was an outstanding athlete and extremely popular, and I was neither. These qualities, being much more important during school years than scholastic achievement, gave me a feeling of inferiority that undoubtedly did all kinds of bad things to my psyche.

One thing this probably did was make me determined to excel at something. My determination may have been reinforced by learning that I scored only 100 on a high school intelligence test. I gathered that 100 was not really a sign of brilliance. This in turn may have been reinforced at some point by my father expressing his opinion that when choosing a career, persons with mediocre talent should not attempt to master a broad comprehensive field, but instead should specialize in some narrow aspect of a field where they might hope to become truly expert. This I have in fact done, although I doubt it was done consciously. And whether the high school IQ score was accurate or not, my father's idea seems to have worked for me.

My father was convinced that public schools were better than private ones for a number of logical reasons. I am sure his children would have gone to public schools anyway, not only for financial reasons, but because it would not be fitting for a prominent public school teacher to send his children to a private school.

At any rate, I went exclusively to public primary and secondary schools and have never regretted it. Most of my teachers were good, and some were outstanding. I remember an exceptionally good physics teacher saying (circa 1925) "I do not know why there should only be 92 elements, perhaps additional ones will be discovered some day." The large class sizes (40 was the norm) did not seem to be much of a disadvantage. Perhaps this discouraged spoon feeding. Standards were high.

I had skipped ahead a number of grades in elementary school, which was

easy in those days. But I had not skipped ahead socially, so I stayed out of school now and then. One semester after finishing elementary school was spent working on an uncle's farm. A year after high school was spent half as an ordinary seaman on a freight boat to the Philippines and Korea, the other half working on another uncle's ranch in Nebraska. These were distinctly maturing experiences, particularly the shift at age 16 from a sheltered religious home environment to that of a tramp ships' forecastle (learning a whole new range of adjectives).

Graduate School

As mentioned above, my first inclination was to combine my brothers' vocations, and thus I enrolled at Northwestern in chemical engineering. But then I spent my sophomore year in Germany at the University of Frieburg with a schoolmate who was a premed (how this came about is not particularly relevant). My companion was so enthusiastic about medicine that I decided I wanted a piece of the action. He suggested that perhaps I should go into biochemistry. He said that so little was known about biochemistry that anything you found out would be new (which was not far from wrong in 1929!). So when we came back, I switched to a chemistry major, and two years later entered the University of Chicago as a graduate student in "physiological chemistry."

My thesis advisor was Frederick Koch, who together with Thomas Gallagher, was trying to isolate the male sex hormone from enormous volumes of urine (every male who came on the premises had to contribute). They assumed that the potency in biological units per mg would be as great as that of the estrogens that Doisy had already isolated. I remember the day (in 1936?) when it was announced that Butenandt had isolated testosterone, and that a unit of activity was much larger in mass than expected, i.e. their preparations were purer than they thought. Whereupon they looked at their best preparations and, in fact, found crystals of the hormone! This was a very blue day in the department.

As a graduate student, I did not have sense enough to pick a thesis project in the field of my advisor. Koch was very tolerant and let me choose for myself. I picked a subject that had something to do with ketone body metabolism, a subject no one in the department knew anything about or had much interest in. After floundering around for a time with some very naive in vitro experiments, I ended up concentrating on the development of a micro method for measuring ketone bodies in one ml of normal blood. This involved the construction of a very complicated homemade multichambered glass distillation apparatus, which permitted delivery from a single volume of blood extract, first acetone itself plus acetone from the degradation of acetoacetic

acid, and second, the acetone from oxidation of β-hydroxybutyric acid. The acetone in the two fractions was determined by an iodometric titrimetric procedure I had modified to increase the sensitivity 10- or 20-fold and decrease the blank more than 20-fold. The procedure worked well in my hands and provided the first reliable values for normal blood levels in the rat. But no one in his or her right mind would ever have used the method, and it was never published.

I believe this atypical graduate program increased my self-reliance and self-confidence and may have been better in the long run than a program designed and monitored by a conscientious thesis advisor. Although in one sense my graduate school research was wasted, my thesis subject did get me hooked on micro methods. I continued to be fascinated all my life with ways to increase analytical sensitivity. This turned out to be for me the specialization that my father recommended for people of limited ability. I had the good sense to recognize that biological analytical methods, micro or macro, were of little value unless they were designed to meet specific needs. Consequently, in most cases my methods were published only in the methods section of papers in which they had been used.

During the second year at the University of Chicago, the Dean asked if I would be interested in working for an M.D. along with a PhD. He pointed out that I already had taken many of the preclinical courses, that he was willing to back-date my admission to medical school, and the quarter system made it easy to squeeze four academic years into three calendar years. M.D.-PhD programs were rare in those days; Chicago was one of the few universities that made such programs feasible. My family was supportive because in the depths of a depression (which this was), an M.D. looked like good insurance. So at a commencement five years after my matriculation, I received two diplomas. When President Hutchens of the "Great Books" fame handed me the second diploma, he asked if he hadn't seen me somewhere before. Although I have never practiced medicine and would not claim that medical training greatly changed my life, I still feel lucky to have received this educational dividend. It has certainly added to my enjoyment of biomedical research, broadened my perspective about living systems, and been good for my ego.

Another dividend of my University of Chicago experience was meeting Baird Hastings and working briefly in his laboratory in Billings Hospital. His attitude about research was that it was an exciting game. There was competition, but it was between friends who were all working for the same goals. One should therefore rejoice in the success of the other fellow. This helped restore my somewhat idealistic concept of research: that the scientific edifice is so grand and so important, that adding even one sound brick to the growing structure is a worthy achievement.

HARVARD

Baird and I hit it off well together, and after graduation I wanted very much to work in his department at Harvard, where he had since moved to succeed Otto Folin. Postdoctoral fellowships were almost non-existent in those days, but the Rockefeller Foundation offered a few, and Baird suggested I apply for one.

I made two alternative proposals, one of which I will describe since it illustrates considerable naiveté and my micro method hang-up. I proposed to confirm directly and measure the relationship between mass and energy, which was still somewhat theoretical. I calculated that if I built a closed glass apparatus containing a liter of bromine and an equivalent amount of sodium that were so situated that the two elements could be made to react slowly enough to dissipate the heat without disaster, that the weight change should be measurable (a few micrograms).

Not surprisingly, the Rockefeller Foundation was not enchanted with this idea nor with the other proposal on a subject that I have forgotten. (As far as I know, no one since then has ever tried to weigh directly a decrease in mass from a large dissipation of chemical energy.)

Fortunately, Baird found money ($2000 per year!) for a job as sub-instructor, which I heard about a month or two before graduation, and which would start immediately thereafter. (My luck continued, although even during the Depression, $2000 per year was not easy to live on, particularly since I was married by this time.)

The research plan was for me to continue one of Baird's basic interests, that of electrolyte metabolism (which involved measurements of Cl^-, Na^+, K^+, Mg^{2+}, and Ca^{2+}), and I offered to develop micro methods that would extend the investigation to milligram-size tissue samples. Perhaps the most useful applications were the studies of electrolyte changes in the myocardium as the result of ischemia (1–3), and in the heart, skeletal muscle, liver, brain, and kidney as the result of aging (4–6). The ischemia study was made in collaboration with Herman Blumgart, the cardiologist. The hypoxia experiments were made with Otto Krayer, Chairman of the Pharmacology Department, who was an expert with the heart-lung apparatus. The aging studies were made in collaboration with Clive McKay of Cornell University, who was able to double the life span of rats by drastically restricting their food intake. (Unfortunately, restricted food intake also delayed their maturation and did not prove to be of much value to rats once they were fully grown.)

A spin-off from the aging study was the development of micro methods for measuring collagen and elastin, which proved useful to a few other investigators (7). [Dorothy Gilligan and I measured these in everything from a rat's aorta to an elephant's ligamentum nuchea (7).]

Advancement at Harvard in those days was rather slow, and hearsay is that this may still be the case. For many years after he became Chairman of Pharmacology, with a worldwide reputation, Otto Krayer was still Associate Professor. It was not until I had been at Harvard for four years and was about to leave that I finally worked my way up to full Instructor. It was therefore not too difficult for my good friend, Otto Bessey, to persuade me to join him at the brand new Public Health Research Institute in New York City where he was to be the Head of the Department of Physiology and Nutrition.

Carlsberg Laboratory

One thing that Baird did for me while I was still at Harvard, and for which I am especially grateful, was to arrange a fellowship from the Commonwealth Fund that permitted me to work for five months with Kai Linderstrøm-Lang at the Carlsberg laboratory in Copenhagen. This was one of the most rewarding experiences of my life. Lang became one of my two scientific idols (the other being Baird himself). World War II began four days after I arrived with wife and baby. Because of the war, fellows from other European countries had to stay home, so the three American fellows (the other two were Paul Zamecnik and Chris Anfinsen!) had almost the full attention of Lang and his colleague Heinz Holter.

Lang was the most talented human being I have ever known. In addition to being a superb investigator (physical biochemist), he played the violin beautifully, sang delightfully, and was a self-taught artist who painted incredibly fine works of art. To top it off, he was intrigued by micro analytical methods and had invented and developed a whole scheme of quantitative histochemistry together with the appropriate devices. The constriction pipette, for example, was invented by Milton Levy while he was a fellow in Lang's laboratory (8).

If I was attracted to micro methods before I went to Copenhagen, I was an incorrigible addict by the time I left.

THE PUBLIC HEALTH RESEARCH INSTITUTE OF NEW YORK CITY (PHRI)

One of the reasons why Otto Bessey, who was a nutrition expert, wanted me to join him in New York was his belief that the studies he envisaged of the biochemical effects of nutritional deficiencies would require new microchemical methods. This belief was reinforced by the fact that the new institute had just opened when the attack on Pearl Harbor occurred. We decided that one of the most useful things we could do for the war effort was to devise a battery of practical blood and urine tests to screen for nutritional deficiency in the general public.

Otto Bessey and I shared equally in the research and the credit from the very beginning. Later, we were joined by Helen Burch, who became a key participant, particularly in the nutritional studies.

Urine tests (for thiamine and riboflavin) were not particularly micro, but the blood methods had to be quite sensitive, and those we devised permitted assay of the plasma from a single, 0.1 ml blood sample (from finger or ear lobe) for vitamin A, carotene, ascorbic acid, iron, total protein, and alkaline phosphatase, this last an index of vitamin D deficiency. We used these methods in a number of New York City high schools, from poor and rich neighborhoods, and on an international study in Newfoundland made before and after flour enrichment. The methods were also widely used by others for studies throughout the United States, and immediately after the war on nutritionally jeopardized populations in Europe. In one instance, when the methods were applied to a large sample of Munich residents, the ascorbic acid levels seemed unreasonably high, considering the acute shortage of fresh fruits and vegetables. Upon further investigation, it was discovered that large quantities of potatoes were being smuggled in from the countryside. Potatoes are an excellent source of vitamin C if they are boiled with their skins on, as was the local practice.

One of our own wartime studies still has considerable nutritional relevance. We collaborated in an elaborate study of ascorbic acid nutrition conducted by the Royal Canadian Air Force with "volunteer" personnel. For eight months, groups were maintained on diets supplying from 8 to 78 mg of ascorbic acid per day. At the end of this period we were invited to measure ascorbic acid in the plasma and in the buffy coat (white cells plus platelets). Measurements were made just before and during realimentation with large amounts of ascorbic acid (9). The results showed that with an average ascorbic acid intake of 23 mg per day, the buffy coat ascorbic acid is maintained at only about half the level attained with 78 mg per day, which in turn is about 90% of that attainable by realimentation with 2000 mg per day for four days. The data on retention during realimentation indicated a maximum body storage capacity of almost 4 g.

Another study, which also concerned vitamin C, was made with four genuine volunteers from our own staff (10). This was an assessment of the effects of ingesting for 90 days what at that time seemed like an excessively large intake of this vitamin: 1000 mg per day in divided doses. These volunteers had been receiving an estimated 75 to 100 mg per day from their regular well-balanced diets. The plasma ascorbic acid level rose an average of 50% during the first day where it stayed for the rest of the time; the buffy coat vitamin level (a good measure of body stores) did not change significantly at any time, and 80% of the 1000 mg intake was promptly excreted in the urine. No adverse symptoms were detected. Thus, vitamin C intakes that are much above what can be obtained on a good diet are promptly eliminated. This may

well be why the enormous doses some enthusiasts recommend (up to 10,000 mg per day) usually do little harm (or good).

We also devised during the war an alkaline phosphatase method, which is still widely used (11). It was based on a study by King & Delory (12) of a wide variety of potential phosphatase substrates, and without our knowledge had already been introduced by Ohmori (13). So we received more credit than we deserved. The substrate, *p*-nitrophenyl phosphate, was originally obtained from Eastman Kodak, but they subsequently discontinued it. One day I happened to sit next to Dan Broida on the train (sic) coming back from a FASEB meeting in Atlantic City. I asked if his small, versatile company might like to make *p*-nitrophenyl phosphate for general use. He agreed and later gave this idea partial credit for getting Sigma Chemical Co. started.

A more famous method that also came out of the PHRI days was our protein procedure, which employs the Folin phenol reagent (14) and is merely a modification of the original 1922 method of Wu (15). We needed a quick and easy method for measuring antigen antibody precipitates from small amounts of plasma of nutritionally deficient rats. We tried the method that had been used for a similar purpose by Pressman (16), and by Heidelberger & MacPherson (17), but could not help tinkering with it, particularly in regard to the Cu^{2+} requirement that was first recognized by Herriot (18).

In the complete absence of Cu^{2+}, color development reflects only the content of tyrosine and tryptophan. The addition of Cu^{2+} gives a major increase in color owing to reaction with some of the peptide bonds themselves. When no Cu^{2+} is added, adventitious Cu^{2+} contamination gives partial, erratic color development, which had given the method a bad reputation.

After moving to St. Louis, we continued to use our modified method without publishing the details, but passed them on to whoever wanted them. This included Earl Sutherland, then in Carl Cori's department. He complained of being tired of referring to "an unpublished method of Lowry." So we finally got down to making a thorough study of the procedure: its limitations and virtues, and the results it gave with different proteins and tissues in comparison with the Kjeldahl method (an analytical headache). The first submission to the *Journal of Biological Chemistry* was returned for drastic shortening. This shortening may have improved the paper, but forced us to omit some details that perhaps would have lessened the plethora of subsequent papers by others describing improvements and precautions.

It may be worth commenting on why this paper, which really was not very original, came to be used so widely in spite of its inherent limitations. I believe this was because most biochemists had to measure proteins; the method was simple, sensitive, and reproducible; and it was used early by two outstanding biochemists who happened to be my friends, Earl Sutherland and Arthur Kornberg.

Another method we developed at PHRI was a colorimetric procedure for measuring inorganic phosphate (P_i) under conditions mild enough not to significantly hydrolyze the more unstable organic phosphates (19). We had already experimented a great deal with modifications of methods to measure P_i with acid molybdate reagents, all of which depend on the fact that phosphomolybdate is easier to reduce (to a blue compound) than molybdate alone. The factors that affect the rate of color development with P_i (as well as with molybdate itself) are molybdate concentration, pH, temperature, and the type and concentration of reducing agent. All but the last also affect the rate of hydrolysis of labile organic phosphates. Herman Kalckar, who was in the Department at that time, was working with ribose-1-phosphate generated by nucleoside phosphorylase (20). This phosphate is too unstable to permit P_i measurement by the classic Fiske & Subbarow method (21) and other modifications thereof. I bet Herman that we could work out a molybdate method to do this. We won the bet, but after more work than expected. We raised the pH from below 1 to 4 and substituted a stronger reducing agent, ascorbic acid.

Along with necessary work in developing and applying specific analytical methods at PHRI, we did a modest amount of work on instrument adaptation. When the Beckman DU spectrophotometer came out, we were among the first to get one and were particularly impressed by it because Otto and I had grown up with visual colorimeters (ugh). Because the standard cells required a wasteful 3 ml of solution, we promptly had a local company (Pyrocell) make special microcuvettes and an adapter that permitted us to use as little as 30 μl of solution without reducing the light path, giving a 100-fold increase in sensitivity (22).

We also had been introduced to fluorimetry because others had found this offered the best modality for measuring riboflavin and the riboflavin coenzymes, as well as thiamine (after conversion to thiochrome). We were delighted with the extreme inherent sensitivity of fluorescence measurement, but unhappy with the low sensitivity of available commercial fluorometers. We therefore replaced the simple phototube of a commercial instrument with a photomultiplier tube and made other modifications to reduce light leaks that were giving intolerably high blanks. The result was a 1000-fold increase in useful sensitivity (23). On the basis of this prototype, we persuaded the Ferrand Optical Company to manufacture a similar instrument, which proved eminently satisfactory, and which has gone through many model changes since then.

QUANTITATIVE HISTOCHEMISTRY

In 1947, I was invited to become Head of the Department of Pharmacology at Washington University in St. Louis. This was quite a gamble on the part of the university. I had never had a real course in pharmacology, nor had I done

any research that was even marginally pharmacological. Moreover, my two predecessors, Carl Cori and Herbert Gasser, were both Nobel Laureates, and there was no sign that I would get to Sweden except as a tourist. At any rate, I was terribly flattered and of course accepted.

This permitted me to return to a deep interest in quantitative histochemistry, which I had acquired in Baird Hastings' Department at Harvard and had been further fostered by exposure to Linderstrøm-Lang and Holter in Copenhagen. I, therefore, immediately applied for support from the Committee on Growth of the American Cancer Socicty for a study of the "Quantitative Histochemistry of the Nervous System." It was obvious that if any part of the body required a histochemical approach, it was the brain, because it is such an incredible mixture of different kinds of cells. Generous support was soon forthcoming and has continued ever since, even though our direct applications to cancer research have been minimal.

The original histochemical approach of Linderstrøm-Lang was to analyze alternate histological sections for the substance of interest and to stain intervening sections to permit quantification of the cell types present. Correlations between cell type and substance were then looked for. This worked well with the tissues that Lang had examined, in which only a few cell types were present, and where the cell proportions changed gradually over a considerable distance. This did not seem appropriate for brain, where more cell types are present and changes can be abrupt, even occurring within a single section. I therefore proposed to make freeze-dried sections, which could be examined at room temperature and from which small identified portions could be dissected out, weighed, and analyzed. This was a modification of a procedure that Chris Anfinsen and I had developed for retina in Baird Hasting's department six or seven years earlier (24), and which Chris had applied to good advantage (25, 26).

I was gambling that substances to be measured, particularly enzymes and metabolites, would withstand freeze-drying and subsequent brief exposure to room air and temperature. Fortunately, stability was not much of a problem, although a few enzymes and such easily oxidized substances as NADH and NADPH did not tolerate more than a few hours in room air. On the other hand, after freeze drying, all components of the sections appeared to be stable indefinitely under vacuum at $-70°C$.

A major advantage of the use of freeze-dried sections, instead of fresh sections, was the preservation of metabolically labile substances at the levels that existed in vivo at the time of freezing.

Instrumentation

To exploit the analytical possibilities with freeze-dried material required appropriate special tools and ultimately much increased analytical sensitivity. The first requirement was to measure the size of the samples dissected out of

the dry sections. The easiest way to do this proved to be by weight, and the simplest imaginable analytical balance proved to be the best. This is merely a quartz fiber of appropriate thickness and length mounted horizontally, like a fishpole, with one free end on which the sample is placed (27). The displacement of the tip is measured on the scale of an eyepiece micrometer of a horizontal dissecting microscope. For larger samples (0.1 μg or more), a small pan of very thin glass or quartz is affixed to the fiber tip. For smaller samples, no pan is needed, since surface forces ensure adherence. The most sensitive quartz fiber "fishpole" balance (made by Takahiko Kato for weighing nuclei of large individual neurons) could weigh 0.1 nanogram samples to 2% (a dry erythrocyte weighs about 0.03 nanograms). The fiber for this balance was about 3 mm long, had a thickness of 0.3 μm, and the tip drooped 0.6 mm under the weight of the fiber itself.

This type of balance is a simplification of an earlier balance that was inspired by studying with Linderstrøm-Lang (28), but actually a quartz fishpole balance was used in 1915 by Bazzoni (29) to prove that musk loses weight in giving off its odor. This fact had been challenged because the loss is so small as to easily escape detection.

To achieve high sensitivity usually requires reducing the analytical volume; otherwise the concentration of the substance measured becomes too low for precision. We have found the best solution with analytical volumes less than 5 μl is to work under oil in small wells drilled in a Teflon block (30). Volumes in the 0.05 to 0.5 μl range are quite manageable.

The clear choice for manipulating small volumes of liquid is the Lang-Levy constriction pipette, which as mentioned earlier was invented by Milton Levy when he was a postdoctoral fellow in Linderstrøm-Lang's laboratory. These pipettes had been shown to be capable of precise delivery in the 1 μl range. Necessity forced us to explore smaller pipettes. It proved possible to make constriction pipettes out of quartz tubing down to 0.000,2 μl volume that still had a precision of 2%. However, we have rarely required those smaller than 0.01 μl.

Unlimited Sensitivity

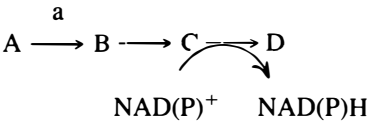
In our earlier attempts to achieve high sensitivity, we used a variety of colorimetric and fluorometric methods, choosing those with the highest absorption coefficients or fluorescence. Later, one development made it easier to design sensitive methods for a wide variety of enzymes and metabolites, and a second development made it possible to increase sensitivity almost without limit. The first improvement was to take advantage of the fact that NADH and NADPH are fluorescent, and that as Kaplan et al showed (31), NAD^+ and $NADP^+$ can be converted into highly fluorescent compounds wth strong alkali. This improvement made it possible to measure with high sensitivity any enzyme, or the substrate of any enzyme, that directly or with

the aid of auxiliary enzymes can oxidize NADH or NADPH, or reduce NAD^+ or $NADP^+$. The technique used is strictly analogous to what had already been done spectrophotometrically by others following the initial lead of Negelein & Haas (32). The difference is that the fluorescence measurements are about 100 times more sensitive than those based on light absorption. Paul Greengard had already had the idea of using pyridine nucleotide fluorescence in this way, and had applied it to the measurement of a number of tissue metabolites (33).

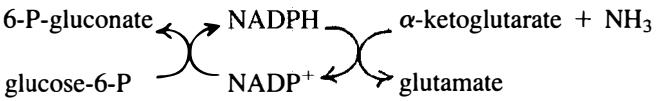
If the reaction is in the direction to produce NADH or NADPH, the fluorescence is measured directly. If the reaction is in the direction to produce NAD^+ or $NADP^+$, the excess NADH or NADPH are first destroyed with acid to which both nucleotides are very sensitive, and then strong alkali is added and heated to produce the highly fluorescent products described. There are few substances of metabolic interest that could not be measured with the aid of an enzyme sequence terminating in a pyridine nucleotide reaction. The versatility of this approach improved as more purified enzymes became commercially available.

What finally gave us all the sensitivity we could use was enzymatic cycling. This technique is an exploitation of enzyme systems to amplify the pyridine nucleotides generated by the specific enzyme reactions just described (34). (Janet Passonneau joined the laboratory about this time and was a key figure in most of the work for the next 10 years.)

The following is an example of an enzymatic cycling amplifier system and its use. The problem is to measure metabolite A or enzyme a:



After the specific reaction, whether a timed reaction to measure an enzyme a or a stoichiometric reaction to measure the metabolite, the excess pyridine nucleotide used to drive the specific step is destroyed with alkali (as in this case), or with acid if the pyridine nucleotide reaction is $NAD(P)H \rightarrow NAD(P)^+$. In either case, the pyridine nucleotide formed is used to catalyze a two-enzyme cyclic reaction, which alternatively oxidizes and reduces the nucleotide, thereby yielding one molecule of the product of each enzyme for each turn of the cycle:



After a sufficient number of cycles (which can be 25,000 per hour or

more), the reaction is stopped, usually with heat; and one of the products is measured (again with an enzyme reaction that yields NADPH or NADH):

$$\text{6-P-gluconate} + \text{NADP}^+ \rightarrow \text{ribulose-5-P} + \text{CO}_2 + \text{NADPH}$$

The yield from 2×10^{-14} mol of initial nucleotide when amplified 25,000 times gives a fluorescence signal that is easily read in a final volume of 1 ml.

Somewhat greater amplification can be achieved by cycling longer [As a stunt, 400,000-fold amplification of NADP was obtained with a three-day incubation (35).] More practical is to simply repeat the cycling step: In the NADP cycling example given, after the indicator step reaction, the excess $NADP^+$ is destroyed with alkali and heat, and the NADPH is further amplified as needed. Two serial 25,000-fold cycles would yield 625,000,000-fold amplification, or sufficient sensitivity to measure about 10^{-18} mol of original sample, i.e. less than a million molecules.

One could imagine further amplification by triple cycling. This we have never tried for several reasons: (*a*) We have never needed more amplification; (*b*) we recognized some difficult problems; and (*c*) we lacked the courage. The biggest problem, even with the degree of sensitivity that can easily be obtained with double cycling, is analytical noise. Only rarely can the concentration of the reagent blank at the initial specific step (i.e. before any amplification) be kept below 10^{-8} M. A good rule of thumb for reasonable precision in any assay is to keep samples at least equivalent to the overall blank. A 10^{-8} M solution contains 10^{-14} mol of the solute in 1 μl and 10^{-18} mol in 0.1 nl.

Some historical perspective on enzymatic cycling may be in order. Although we have substantially refined and exploited this powerful tool, we did not invent it. Warburg et al (36) were the first to use the cycling principle for measuring NADP ("TPN"!) with a system containing glucose-6-phosphate and the "old yellow enzyme." Although they obtained 330 cycles in 10 min, because the signal was O_2 consumption measured manometrically the sensitivity was not great.

Jandorf et al (37) used the cycling principle to measure NAD in a system containing the enzymes needed to convert fructose-1,6-bisphosphate to glycerolphosphate and phosphoglycerate with the release of CO_2 from bicarbonate buffer. The NAD functioned to alternatively oxidize glyceraldehyde-3-phosphate and reduce dihydroxyacetone-phosphate. The cycling rate was about 1300 per hour. By the use of this system in the Cartesian diver (an analytical exploitation due to Linderstrøm-Lang), Anfinsen was able to measure with precision as little as 2×10^{-12} mol of NAD (38). More recently, Glock & McLean (39) obtained 30- to 50-fold enzymatic cycling of NAD or NADP with cytochrome *c* and either alcohol dehydrogenase or glucose-6-

phosphate dehydrogenase. Useful enzymatic cycling systems for compounds other than NAD or NADP have been devised by other investigators as well as ourselves (40).

ENZYMES AND METABOLITES

The glycolytic pathway consists of a long series of enzymes, which in average brain differ 100-fold in potential activity. And yet in a steady-state situation, the net flux through every enzyme step must be the same (ignoring side reactions). For example, during peak glycolytic flux in mouse brain, 50% of potential aldolase activity is used, but only 0.5% of that of phosphoglycerate kinase.

In each case, the kinetic properties of the respective enzymes, together with the steady-state levels of their substrates and products (plus the concentrations of any other effectors) must yield the same net velocities. Obviously, a full understanding of a metabolic system involves a great deal more than knowing just the levels of the enzymes concerned.

All our early histochemical studies concerned only the tissue distribution of enzymes, particularly enzymes of energy metabolism. This is because it takes much less sensitivity to measure the activity of an enzyme than the concentration in a tissue of its substrate or product. Brain lactate dehydrogenase can produce in vitro several moles of lactate per kg per hour, whereas the brain lactate concentration is normally only about one mmole per kg. But, as suggested, there is good reason to measure both the enzyme and its metabolites. The enzyme measurement indicates the capacity to carry out the metabolic reaction, whereas the levels of substrate and product of that enzyme, taken together with the flux, can indicate its actual function under the conditions of observation. Therefore, with the major increase in sensitivity at our disposal due to the substitution of fluorometry for spectrophotometry, Janet Passonneau and I decided to measure the levels in whole brain of all the intermediates of the glycolytic pathway plus ATP and phosphocreatine under control conditions, and during the sixfold increase in glycolysis that results from total ischemia (41).

Mice were decapitated and the heads frozen at intervals from 3 seconds to 10 minutes. Mice were used because the small head size minimizes artifacts from the delay in freezing the deeper portions.

During the first few seconds, fructose-6-phosphate fell and fructose-1,6-bisphosphate rose dramatically together with the other metabolites below it in the pathway. These results clearly indicated a control point at the phosphofructokinase (PFK) step, which was activated by the consequences of the lack of oxygen. [Cori et al (42) had previously concluded that PFK is a control step in muscle.] The changes in the other metabolites indicated the absence of any

other important control step between glucose-6-phosphate and lactate. However, the first step in glucose metabolism was clearly also a control point, but the data did not permit distinction between control by hexokinase from control by glucose transport into the cells.

A companion in vitro study was made of the maximal activities and kinetics of all the enzymes of the glycolytic pathway in mouse brain homogenates under conditions simulating the pH, ionic strength, and temperature of brain (43).

Putting together the data on enzyme capacities and their kinetic properties, with the differences in the levels of the substrates and products of these enzymes under two different glycolytic fluxes, permitted a much better picture of the logistics of this important pathway. It helped to explain, for example, why some enzyme levels (expressed in terms of their maximum capacities) had to be much higher than those of others.

A full discussion of these results would go beyond the present purpose. However, I would submit that assessment of metabolite levels and their changes under various circumstances of interest can be a very informative approach, which has been rather underutilized.

PHOSPHOFRUCTOKINASE

Although study of biological problems requiring high analytical sensitivity has constituted the theme of most of our research, we have had several major distractions. One of these involved phosphofructokinase (PFK). Our first encounter with this remarkable enzyme was simply concerned with setting up optimal, reproducible, stable conditions for measuring it in brain. As usual, we tested different buffers, and to our surprise found not only that a phosphate buffer was by far the best, but also that without phosphate, activity was very low, and accelerated remarkably during the assay, as it was being followed in the spectrophotometer. We had stumbled onto what was later designated an allosteric phenomenon, and weren't smart enough to realize it. In the absence of P_i, the reaction was severely inhibited by ATP, as Lardy & Parks had discovered (44); but as the reaction proceeded, ADP accumulated and probably some of the fructose bisphosphate generated was not removed fast enough. These two PFK products, both deinhibitors of PFK (45), were probably sufficient to overcome the ATP inhibition. In any event, we did not pursue this exceptional opportunity, and about this time or soon after, Pardee discovered (1956) the feedback inhibition of aspartate carbamoyl transferase by CTP, probably the first clear-cut example of allosterism (46).

We did, however, go back to PFK later, after observing its dramatic activation in brain during ischemia. We were able to report that the kinetic properties of PFK made it perfectly suited for controlling glucose metabolism according to need (45). PFK is inhibited by ATP, which falls in ischemia, and

this inhibition is overcome by fructose-6-phosphate, fructose-1,6-bisphosphate, ADP, AMP, P_i, and NH_4^+, all of which usually increase during ischemia.

Soon afterwards, we discovered that citrate is another potent inhibitor of PFK, and that its action is synergistic with ATP (47). This discovery was rather exciting, because it meant that the citrate cycle can feed back to control the glycolytic pathway. Recently we learned that Neifakh et al in 1953 had already reported in the Russian literature that citrate is a PFK inhibitor (48).

We have come to regard PFK as "the most complicated enzyme alive." In addition to the effectors already mentioned, Uyeda & Racker found that phosphocreatine and 3-phosphoglycerate are negative effectors (49), Krzanowski & Matschinsky reported that 2-phosphoglycerate, 2,3-bisphosphoglycerate, and phosphopyruvate are all potent negative effectors (50), Mansour & Mansour showed that cyclic AMP is a positive effector (51), and Van Schaftingen et al (52) found that a previously unknown metabolite, fructose-2,6-bisphosphate, is probably the most potent positive effector of all (52). Many of these effectors interact in a synergistic way, probably indicating a multiplicity of allosteric sites (53).

APPLICATIONS OF QUANTITATIVE HISTOCHEMISTRY

As mentioned earlier, our original purpose in trying to extend the quantitative histochemical approach of Linderstrøm-Lang & Holter was to determine the composition of different parts of the brain. However, practically every organ and tissue is heterogeneous, not only in regard to the types of cells present, but often in regard to the composition of any given cell type. Let me briefly review some of the aspects of this heterogeneity that have been explored by ourselves and others.

Linderstrøm-Lang & Holter, using their original approach, made some classical studies of the gastric and intestinal mucosas, and found, for example, that gastric chief cells are the source of pepsin (54). This approach has been used by Alfred Pope in several studies of the different layers of the cerebral cortex (55). David Glick has made a wide range of important applications of his own adaptations of the Linderstrøm-Lang approach (56). Of particular importance are his comprehensive studies of the different layers of the adrenal gland. Giacobini has been especially active in studies of the metabolism of single neurons, making use of an ultrasensitive adaptation of the Cartesian diver (57).

Nervous System

Investigations of the nervous system from our laboratory started with measurements of metabolic enzyme levels in 0.1 to 1 μg samples of specific

layers of such structures as the cerebellum (58), hippocampus (59), and retina (60), and finally progressed to the 1- or 2-nanogram level with large single neurons (61) and even their nuclei (62). One study was made with Janet Passonneau on the effect of ischemia on ATP, phosphocreatine, glucose, and glycogen in single neurons from the spinal cord anterior horn and the dorsal root ganglia (63). This study involved measurements at the 10^{-15} mole level.

One of the most impressive brain histochemical studies was made by Takahiko Kato (64). He dissected out eight different types of neuron cell bodies from freeze-dried sections, with dry weights ranging from 0.2 to 10 ng, and analyzed them individually for one of seven different enzymes of the glycolytic pathway and citrate cycle. As an add-on (65), he measured the distribution of nine enzymes between nucleus and cytoplasm of individual dorsal root ganglion cell bodies.

Kidney

Each kidney nephron consists of a chain of structures with very different functions and with very different enzyme and metabolite compositions. This makes the kidney an ideal candidate for quantitative histochemical exploitation. I believe the first kidney study along this line was reported in 1956 by W. Peter McCann, a postdoctoral student in this laboratory (66). This was soon followed by a renal paper by Dubach & Recant from the Department of Medicine (67) and one by Kissane from the Department of Pathology (68). Somewhat later Dr. Helen Burch began a long series of very fruitful investigations of quantitative renal histochemistry, which continued for almost 15 years until her death in 1987 at 80 years of age. Meanwhile, this approach to renal biochemistry and pathology spread outside this institution, first to the University of Illinois Medical School in Chicago through the interest of Bonting & Kark (69), and then on a larger scale to Switzerland and Germany, mainly through the influence of Dr. Dubach, who is now Professor of Medicine in Basel.

Skeletal Muscle

For many years, biochemists treated skeletal muscle as though it were a homogeneous tissue. However, when enzyme-staining methods were applied, this was found to be far from true. Credit for the first quantitative enzyme measurements of single muscle fibers goes to James Nelson, then in the Department of Pathology at Washington University. He found large differences in the levels of glycogen phosphorylase among fibers from the same muscle (70). In 1975, a Swedish group began to apply quantitative enzyme methods to individual freeze-dried muscle fibers. Instead of making sections of the frozen muscle, Essén et al (71) freeze-dried a portion of the muscle and then dissected out segments of intact fibers several mm long. When a little

later, we could not resist joining in a medical school–wide muscle program, we adopted this fiber isolation procedure. It proved to be quite feasible to analyze single fiber segments 2 or 3 mm long for many different enzymes and/or metabolites. For any particular assay, samples weighing 10 to 20 ng (10 to 50 μm in length) were simply cut off one end of the fiber, and the rest of the fiber returned to cold storage under vacuum for future use. This made possible direct comparisons between the levels of many different enzymes within the same fiber. The advantage of this was apparent when it was found that among fibers from a given muscle, the ratios between an enzyme of glycogenolysis and one of the citrate cycle might vary 30-fold or more (72). Similarly, it was possible to compare metabolite levels in single fibers from stimulated muscle with the relevant enzymes of the same fibers (e.g. malate with malate dehydrogenase) (73).

MAMMALIAN OVA

To me, one of the most satisfying applications of our microchemical methodology has been to the study of individual ova—first mouse ova and very recently human ova. In 1974, Elizabeth Barbehenn, then a graduate student, and Raymond Wales, a visitor from Monash University in Australia, with major experience in culturing mouse ova, decided to tackle an interesting puzzle: why fertilized mouse ova, before the eight-cell stage, cannot grow with glucose as the sole carbon source, but can do so if pyruvate or lactate is substituted.

The experiments were simple: ova from superovulated mice were starved for 60 min (that is, placed in medium with no carbon source), and then re-fed for 15 min with glucose or pyruvate or both. Ova were freeze dried before and after starvation and after refeeding, and then individually analyzed for glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-bisphosphate, citrate, or malate. The metabolite results clearly showed that before the eight-cell stage, there was a block at the phosphofructokinase (PFK) step (74). The mechanism appears to be that the level of ATP (i.e. a potent PFK inhibitor) is high, and that the level of fructose-6-phosphate is too low to overcome the inhibition. The low fructose-6-phosphate is attributable to a low level of hexokinase, which in competition with highly active glycogen synthase cannot maintain an adequate level of the glucose-6-phosphate:fructose-6-phosphate equilibrium mixture. By the eight-cell and morula stages, there is a sufficient increase in fructose-6-phosphate to overcome the block. The fructose-6-phosphate increase in turn is probably due to the rise in hexokinase known to occur at this time. The biological importance of all this appears to be that with both glucose and pyruvate available, pyruvate satisfies the energy

requirements, and glucose is diverted into glycogen to build up a reserve for the implantation process.

This study only required about 1000 ova from 50 mice, and would probably have required ova from many thousands of mice with more conventional methods.

The human ova studies were initiated 15 years later. The impetus came from two sources. Two daughters-in-law and a neighbor's daughter were attending in vitro fertilization clinics without success. During this same period, I reviewed a grant application from Henry Leese from the University of York for funds to support metabolic studies of human ova obtained from the famous Edwards and Steptoe Clinic. (Leese was using the highly sensitive microchemical techniques developed at Harvard Medical School by Claude Lechene.)

I was faced with an ethical dilemma. On the one hand, the success rate of in vitro fertilization was (and is) exceedingly poor, possibly owing in part to the fact that the in vitro incubation media were designed for optimal growth of mouse ova. The reason for this design choice is that practically nothing was known about the metabolism or growth requirements of human ova. We had the tools to at least find out if there are major metabolic differences between the two species, and felt almost obligated to apply these tools. On the other hand, the idea for us to get involved had come from my reviewing a privileged grant proposal.

We solved the dilemma, as far as our consciences were concerned, by writing to Dr. Leese describing the situation and stating that we were going ahead, but would keep him in touch with what we were planning and doing, and would share our results with him before they were published. I half expected an angry letter in return. Instead, I received a most cordial response and a welcome into this field, which only he and Claude Lechene (besides ourselves) had the tools plus the inclination to investigate.

Problems with Federal Support of Research on Human Ova

But solution of our own ethical problem did not mean that an ethical problem of a different sort might not be raised by others. With start-up funds contributed by our own Department and discarded human ova plus normal mouse ova, both kindly made available by Dr. Ronald Strickler of Washington University from the in vitro fertilization clinic under his control, we were soon able to compare metabolic enzyme levels in ova from the two species. Because of the severe limitation in numbers of available human ova, we modified our methodology to permit each ovum to be assayed for as many as 8 or 10 enzymes, or for 4 or 5 enzymes plus as many metabolites. Data were obtained for 17 enzymes of 8 metabolic pathways that demonstrated some dramatic species differences. For example, enzymes of fatty acid

metabolism were as much as 15-fold higher relative to size in human than in mouse ova (75). A variety of data, including the levels of ATP and phosphocreatine, indicated that the limitation of our assays to discarded human ova did not invalidate the results.

With these data, we applied to NIH for funds, and after a little backing and filling, received approval with a very high priority score.

And then the trouble began. It was ruled (as I was told) that before funding, the application "had to go through the Ethics Committee." It was later revealed that there was no Ethics Committee and had been none for eight years! This was in the spring of 1988. Because of our high priority rating and what would appear to be a negligible ethical problem, the NIH decided to use this as a test case to clear the way for other research proposals concerning preimplantation human embryos. Subsequently, the Department of Health and Human Services agreed to appoint an ethics committee, but as of the fall of 1989, there is still no action, and the outcome for the near future in the present political-judicial climate seems dim. Fortunately, nonfederal funds have been granted for two years, and we hope to achieve something useful for in vitro fertilization before those funds run out. (My father never told me that hyperspecialization might get me into trouble.)

2-DEOXYGLUCOSE TO MEASURE GLUCOSE METABOLISM

In 1977, Sokoloff et al introduced the use of ^{14}C-2-deoxyglucose to measure the regional glucose metabolism of brain (76). This radioautographic method is based on the fact that although 2-deoxyglucose (DG) is phosphorylated by hexokinase in parallel with glucose, the 2-deoxyglucose-6-phosphate (DG6P) that is formed cannot be further metabolized along the glycolytic pathway. It therefore accumulates as an index of glucose metabolism. This method has been widely used and has yielded very valuable results. However, it has one important disadvantage. Because the radioautograph cannot distinguish ^{14}C-DG from ^{14}C-DG6P, it has been necessary to wait 30 to 45 minutes after DG injection for the brain DG to largely dissipate before preparing the brain for the radioautographic procedure. This limits the method to studies of long-term events, whereas many brain events of interest take place on a time scale of a few minutes or less.

When we recently found that DG and DG6P could be separately measured enzymatically with $NADP^+$ as cofactor, we realized it would be possible to use the principle introduced by Sokoloff et al to assess brain glucose metabolism on a time scale of a minute or two (77). Moreover, we could employ enzymatic cycling to give the sensitivity needed to study very small brain regions, even down to the level of single neurons.

So far, we have been mainly perfecting the analytical procedures and exploring the new use of DG with whole mouse brain and brain slices incubated in vitro. The methods depend on the fact that DG6P is oxidized by glucose-6-phosphate dehydrogenase, but at a rate 2000 times slower than with glucose-6-phosphate itself. This rate difference can introduce problems, for example, with enzyme impurities. However, these and other problems are manageable, and we are confident that this method of assessment of rapid changes in brain glucose metabolism will prove a useful additional way to study the quantitative histochemistry of brain.

BIOCHEMISTRY: 1932–1990. A PERSONAL VIEW

I have enjoyed almost 60 years of participation in this greatest game on earth and hope to continue a while longer. I feel much the same way about biochemical research that my mother, a fine artist, felt about painting. She said an artist ought not to complain about the poor financial rewards, because the pleasure in making the painting is reward enough. I tried to promote this idea around the laboratory on pay days: "You have all this fun and get paid too!" (But somehow this was never much of a substitute for better pay.)

One of the things one is supposed to acquire with age is wisdom. So I assume my final duty in writing this chapter should be to think of wise things to pass on to future generations. Unfortunately, in spite of much thought, I have come up with very few words of wisdom. So let me instead simply touch on three topics that seem to me particularly impressive concerning the biochemical achievements of the past 58 years.

The Rapid Rise of Biochemistry Since 1932

The changes in biochemistry in 58 years have been astounding. In 1932 many of the vitamins had not been identified, nor had their structures been determined. The accepted structure of cholesterol was incorrect. Only a few of the hormones had been isolated. Relatively few enzymes had been purified and only one (urease) crystalized. "Yeast" and "thymus" nucleic acid had not yet become RNA and DNA, and their functions were completely unknown, but for sure they had nothing to do with genetics or protein synthesis. The members of the Embden-Meyerhof pathway had been identified, but the citrate cycle was still being worked out, and the pentose pathway was unknown. ATP was known to have something to do with muscle contraction, but its broader function had not been realized.

Biochemical progress subsequent to 1932 was remarkably rapid considering the relatively few investigators, that most of them carried heavy teaching loads, and that the amount of financial support was minimal. In 1937–1941, Baird Hastings' whole department at Harvard had one modest outside research grant, two technicians, and I believe $2000 per year from the school for supplies and equipment.

World War II interrupted much of the pure research. However, applied biochemical research, which was supported quite well with federal funds, stimulated the development of improved tools and techniques that paid off well after the war.

The war also convinced influential persons in and out of government that biomedical research, biochemistry included, was a good investment. In consequence, a program of federal research support was instigated that soon expanded to a size no one would have believed possible. (Private foundations also joined in, with the American Cancer Society taking the lead.)

More research required more researchers. The war had turned the consensus around in regard to the intellectual potential of the average citizen. In 1932 it was generally held that only a minority of the population could benefit by a college education, and of these only rare individuals had the special talent needed to do worthwhile research. Both these views proved fallacious (although this elitist attitude is unfortunately not completely dead).

We now have enormously more investigators than in 1932. The increase is in all categories: brilliant, good, and poor. I doubt if the ratios between these categories are much different than in 1932, and the increase in output of high-quality research, by anyone's yardstick, has been sensational.

Biological Aids to Biochemical Research

It is remarkable how much this phenomenal progress in biochemical research has been dependent on the use of natural tools offered by biology itself. Before my day, bioassays were of necessity used to follow the purification of vitamins and hormones. These bioassays usually required measurements with whole animals, and progress was slow and tedious. Later on, bioassays with isolated organs were introduced for rapidly acting substances ranging from epinephrine to prostaglandins and atriopeptins. This type of assay reached its highest sophistication with Vane's organ cascades. Microbiologists made great use of bioassays in the isolation of growth factors for bacteria. And a spin-off was to turn this around and use growth or acid production of bacterial cultures to measure the levels of specific substances in tissue extracts.

I have already stressed the importance of enzymes for measuring other enzymes and their metabolites and cofactors, as well as in participating in enzymatic amplifier systems. But it is not just the convenience and sensitivity that are important. What is really invaluable is the specificity conferred by the use of enzymes as reagents. Whole branches of biochemical investigation would slow down to a snail's pace if it were not for the use of enzymes to cleave proteins and nucleic acids at specific sites, as well as to add specific fragments according to plan. Antibodies, both monoclonal and polyclonal, have similarly proved to be powerful biological tools for biochemical research.

To generalize, the very nature of biological systems to carry out innumer-

able, highly coordinated, synthetic, degradative, and identification functions requires machinery that the skillful experimenter can turn around to unravel the systems themselves. Biology supplies the keys to unlock its own secrets.

The Revolution in Biomedical Categories

In 1932 and for many years thereafter, preclinical medical departments were strictly segregated not only with respect to teaching, but with few exceptions with respect to research as well. Each subject was designated as a separate "discipline," which aptly indicated the strict party lines then existing. Washington University Medical School broke ground when it appointed Carl Cori in 1931 to be Head of Pharmacology, but it was several years before he was accepted into the American Society for Pharmacology and Experimental Therapeutics. Similarly, Cori had to make somewhat of a fuss to get me into ASPET when I took his place in 1947.

One of the most pleasant transitions (even revolutions) in the biomedical world has been the blurring of party lines, first in research, and more gradually in teaching. I hope I am not being a chauvinist by pointing out that this transition was due to a gradual realization that biochemistry in fact pervades every biomedical discipline. How can a cytologist do research or teach without considering the biochemical nature of the cells, or a physiologist investigate secretion or nerve transmission without taking account of the biochemical elements involved? And so on.

When the term "molecular biology" was first introduced, I thought it was somewhat silly, since biochemists had been studying the molecules of biological systems since the late 1800s. I now realize it was a face-saving device for physiologists and biophysicists who had discovered biochemistry and wanted to apply it without seeming to cave in.

In any event, party lines have largely come down, much to the advantage of biomedical research and teaching.

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