1959 Ernst Bischoff Lecture: Adventures In Clinical Chemistry

Oliver H. Gaebler
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During my career in biochemistry and clinical chemistry, three areas of activity have been sources of great satisfaction. First among these is our usual function of developing and supervising chemical laboratories that aid the medical profession in diagnosis, prognosis, and treatment of disease. During 3 years with Prof. V. C. Myers, at the State University of Iowa, and 19 years with Dr. F. W. Hartman, at Henry Ford Hospital, this was my principal assignment. Research was a secondary, but by no means impossible or neglected, pursuit; in fact, many research interests that continue to intrigue me developed during those years. Much of the work in clinical laboratories can be classified as development, for it involves adapting known principles and procedures to practical use; but it also brings one into contact with many challenging problems.

Even prosaic analytical duties may become exciting. One day at Iowa City, everyone had diabetes. All blood sugar reports were held up while telephones jangled and fermentation began at the clinical end of the line. Without my knowledge, but with the best of intentions, the division of analytical chemistry had made our laboratory a present of expensive and highly extracted filter paper, which students in "quant" refused to buy at 2 cents a sheet, because some sheets broke when folded. It was well known at the time that some of the cheapest grades of filter paper contained only traces of reducing substances and prohibitive amounts of phosphate, while some expensive grades were free of phosphate and full of reducing substances.

Although not our principal function, identification of poisons, drugs, and ordinary compounds available at the grocery or hardware store is often expected. While this can be a waste of time or a frustrating venture, I can recall many instances in which it disposed of unnecessary anxiety, exposed fraud, or even established the cause of death. Knowing something about the circumstances may be either helpful or misleading. An alkaline salt from a sack in the garage is obviously available in sackfuls; our sample was sodium carbonate, and nothing to cause alarm. But the white powder which the life of the party took at 3 a.m. was not sodium bicarbonate, as he supposed. It was lead arsenate, apparently selected with indifference from the available packages. Most of the arsenic appeared in the urine, most of the lead in the feces, and results of the mistake were not too serious. Another preparation, thought to be apomorphine, turned out to be tartar emetic, the toxic effects of which are cumulative; it should not have been put into a husband's coffee repeatedly to cure him of alcoholism. A brand new distemper remedy, supposedly imported from Europe, was pure lactose. Panaceas for human ills can usually be disposed of without analysis. If analysis is demanded, it is a good rule to look for ingredients that are more or less harmless and inexpensive.

A second field in which I have been privileged to participate is teaching. In this connection, it might be wise to consider a situation that we may face in the near future. In some highly respected departments of biochemistry, candidates for the Ph.D. degree are no longer required to learn blood and urine analysis. Why should they clutter up their minds with “old stuff” when there is such a wealth of recent knowledge they must have if they are to survive as biochemists? Medical students, on the other hand, are taught blood and urine analysis and are introduced to a great deal of new material. So the M.D. of the near future will have background information to set him thinking, and should be ready to consider recent as well as long-established developments when he gets to the clinic. This is as it should be.

Should the prospective Ph.D.’s in biochemistry be denied contact with our field that might be beneficial to it and to them? Should they be warned against clinical chemistry as a purely routine pursuit in an area where chemists or biochemists who enter will be devoured by dragons? Such attitudes are not realistic. Not long ago, the head of a well-known university department of biochemistry said to me: “I could replace any other member of my staff on short notice, but not the clinical chemist.” Perhaps clinical chemists who are willing and able to teach, and who know the value and limitations of their field, could be useful in this connection.

The theme that men in fields of applied science should participate in teaching is receiving considerable attention in professional journals. An opportunity to do so came to me in 1934, when the chemistry department of Wayne University (now Wayne State University) asked me to start courses in general biochemistry and biochemical methods of analysis. After devoting evenings to this for many years, I consented to fill an unexpected vacancy in physiological chemistry, with intermediary metabolism as my lecture topic — for the next 7 years. Many treasured associations with faculty and students resulted, particularly with the eight candidates for the M.S. degree and 5 for the Ph.D. degree, who completed their research requirements under my supervision.

My third topic is research. During one of his frequent visits to our hospital, the late Charles F. Kettering commented that he and his associates did a lot of research that was published in the form of better automobiles. Many of us must do a great deal of investigation of this type, but if one examines more formal publications it can be seen that quite a few are in three very interesting areas: isolation and identification of compounds, methods of analysis, and metabolic problems. Studies I have selected for discussion are in these areas.

A stimulating and provocative study by Behre and Benedict, which appeared in 1922, presented strong evidence for the absence of creatinine from normal blood. From the Folin picric acid blood filtrate, kaolin did not remove the apparent creatinine, while creatinine added to blood was transmitted to this filtrate and removed by kaolin. My first paper on the topic of isolating creatinine begins with an erroneous statement: “The use of kaolin in removing creatinine from dilute solutions is disadvantageous in that the absorbed creatinine can not be released again for identification.” Benedict later told me that creatinine can be released from kaolin with
magnesium oxide suspension, and that this treatment also releases a chromogenic substance from kaolin with which a Folin picric acid filtrate of normal blood has been shaken. Kaolin was, however, a bulky adsorbent.

In my first isolation procedures, creatinine was concentrated from blood filtrates by adsorbing it on the potassium salt of a new complex acid2,3 which we called picrophosphotungstic acid, or on Lloyd's reagent4,5. The base was then isolated as creatinine potassium picrate. This was converted almost quantitatively to creatinine zinc chloride, which was analyzed for nitrogen content per milligram of creatinine. Thus identification rested upon preparation and partial analysis of two derivatives. Linneweh6 modified one of our procedures, obtained the gold salt of creatinine from beef blood, and submitted it to elementary analysis.

In subsequent studies6,7 we used serum. Initial dilution was avoided by ultrafiltration, since precipitation technic had meanwhile been improved so that creatinine could be precipitated directly from ultrafiltrate. Progress in precipitating creatinine was the result of the activities of a series of investigators (Table 1). The solubility of creatinine potassium picrate in water, which Jaffe8 recorded, calculates to 33.4 mg. of creatinine per 100 ml. Morris9 reduced this to 5.0 by saturating the supernatant with picric acid. By adding an excess of potassium, Gaebler and Keltch9 reduced it to 3.0, or even to 0.6, in initial precipitations in which contamination with potassium picrate did not matter. Greenwald and Gross10 studied the solubilities of double picrates of creatinine with potassium, rubidium, and cesium, and found the one with rubidium to be least soluble. By utilizing this information, and carrying out precipitations in the refrigerator, we found that known creatinine could be precipitated so completely that less than 0.1 mg. per 100 ml. remained in the supernatant.

With this technic, creatinine was readily precipitated from ultrafiltrates of normal human, dog, beef, and pig sera1. In isolation experiments on animal sera, we used an ultrafilter that produced 200 ml. of ultrafiltrate from 300 ml. of serum in 2 hours. Creatinine from beef serum and pig serum was readily accumulated and identified1. Creatinine from beef blood, dog blood, blood and transudate of human nephritics3, and dog blood obtained during experimental retention of various types5,14 had been identified previously.

While these results were very tangible and gratifying, they did not completely satisfy my curiosity. Our first isolation procedures3, when applied to normal blood, were inefficient enough to avoid serious conflict with evidence for absence of creatinine from normal blood.1 As our isolation methods improved,4 our troubles increased, and when we began to wonder about the validity of the kaolin experiment, it turned out that our precipitation method, under suitable conditions6,11, could also be used to precipitate added creatinine without precipitating the creatinine of normal blood. To this day, I have the feeling that the story of creatinine in normal blood is not fully known.

It is, indeed, interesting that induced enzyme formation appeared on our horizon during the resolution of this problem. Miller and Dubos12 used a creatinine-destroying enzyme, formed by soil bacteria grown on a medium in which creatinine
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Table 1

Conditions Affecting the completeness of Precipitation of Creatinine as a Double Picrate.

<table>
<thead>
<tr>
<th>Creatinine potassium picrate</th>
<th>Solubility of creatinine (mg./100 ml.)</th>
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<tbody>
<tr>
<td>In water (Jaffe, 8)</td>
<td>33.4</td>
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<tr>
<td>In saturated picric acid (Morris, 9)</td>
<td>5.0</td>
</tr>
<tr>
<td>In saturated picric acid, with potassium ion in excess (Gaebler &amp; Keltch, 3)</td>
<td>3.0:1 1.21:1 1.36:1 1.51:1 3.0:1</td>
</tr>
<tr>
<td>Rubidium creatinine picrate, (Greenwald and Gross, 10)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>In saturated picric acid, with Rb ion in excess temp. 5.0°C. (Gaebler, 6)</td>
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supplied the nitrogen, as a means of securing evidence for presence of creatinine in blood. However, creatine was thought to be present as such in muscle from 1835 until 1929, when Fiske and SubbaRow described their isolation studies on creatine phosphate, existence of which could have been missed by a variety of “specific” methods.

Of several ventures into the field of analytical methods, determination of bromsulphalein best illustrates the difference that appearance of photoelectric colorimeters and spectrophotometers has made in our work, and the problem that differences in design of the various instruments created.

Figure 1
Absorption spectra of bromsulphalein and bilirubin in 0.05N sodium hydroxide.
Bromsulphalein (BSP) was formerly determined with a test tube comparator obtainable from the sellers of the indicator. That the color of alkaline BSP in icteric sera could not be matched with the BSP standards was mentioned in several papers. When examined in a Beckman spectrophotometer, the absorption spectra of alkaline solutions of bilirubin and BSP are about as different as possible (Figure 1). Although the eye is an amazing optical instrument and detects differences in wave length as well as intensity, its “band pass” is arranged for outdoor purposes. Differences in the band pass of various instruments also create a problem.

In one of our two procedures, we applied two-color spectrophotometry, which enabled us to use a water blank, and reduced the amount of serum required to 0.5 ml. BSP concentration \( X \) was calculated from the absorbancies \( A_580 \) of the solution at 580 m\( \mu \) and 620 m\( \mu \) as follows:

\[
X = \frac{A_{580} - K_1A_{620}}{0.0715 \left\{ \frac{1 - K_1}{K_2} \right\}}
\]

The absorbancy of BSP, per milligram, at 580 m\( \mu \), with dilution, cell depth, and other conditions as specified, was 0.0715. In the spectral region between 620 and 580 m\( \mu \) effects of light scattering by normal or slightly turbid serum, and absorption by hemoglobin or bilirubin, were small and similar. \( K_1 \) was the ratio of absorbancy at 580 to absorbancy at 620 m\( \mu \), deriving from this composite source; \( K_2 \), the absorbancy ratio for BSP at same wave lengths.

Difficulties with this procedure arose when the Coleman Junior Spectrophotometer, with a band pass of about 35 m\( \mu \), was used. \( K_2 \) was 14.3 when absorbancies were determined with the Beckman instrument, which has a very narrow nominal band width, and 3.7 when the Coleman Junior was employed. Small differences in the average value of 1.2 for \( K_1 \) were unimportant when \( K_2 \) was large, but significant when \( K_2 \) was small. It was necessary to choose different wave lengths for the Coleman Junior, or to use the alternative method of calibration given in our paper (Eq. 2, Ref. 14). Newer procedures for determination of BSP have appeared and reference to my procedure has been made to illustrate certain principles.

Since turbidity correction entered into two of our methods, I may say that the linear relationship between log of the absorbancy due to turbidity, and the negative logarithm of wave length has been confirmed with a number of Beckman instruments. It does not apply for wave lengths in the near infrared, or when instruments with wide band pass are used. The best correction for all interferences that can be avoided is to avoid them. If turbidity can not be avoided, one has a choice between correcting for it by a method such as that which we used, or of decolorizing the substance to be measured and subtracting the resulting blank.

My interest in metabolic studies began while I was a candidate for the Ph.D. degree at Toronto, and has been a continuing one. In their pioneer work on gigantism in rats, Evans and Long used alkaline extracts of beef anterior pituitary. Putnam, Benedict, and Teel used it in simulating acromegaly in dogs. Teel salted out the globulin fraction, which contained several anterior-lobe hormones. Such preparations
were made available to me by members of the research staff of Parke, Davis & Company. My first experiments, carried out in 1932, were so striking (Fig. 2) that I soon developed a long-term interest in metabolic effects of anterior-pituitary hormones. When a dog receiving a constant amount of food increased its weight by 1 1/2 lb. within 48 hours and stored quantities of nitrogen, in spite of an increase of more than 40 per cent in basal metabolic rate, it seemed to be high time to investigate! It was well known that a similar increase in metabolic rate induced by intravenous thyroxin is accompanied by marked losses of weight and nitrogen. There was, as usual, nothing thermodynamically illegal about the behavior of our dog. The fall in nonprotein respiratory quotient was due to greatly increased oxidation of fat, not failure to oxidize carbohydrate. Available carbohydrate apparently ran low during the post-absorptive period, since all of the extra calories could be supplied by carbohydrate if a sufficient amount was added to the diet when the anterior pituitary preparation was given.

Nor was storage of nitrogen spurious. The nonprotein nitrogen of the blood, as previously reported by Teel and Watkins, fell during such experiments, and we found no change in fecal nitrogen. So began a long-term program with which we are still occupied. Effects of growth hormone and corticotropin on nitrogen metabolism, and the relationship of insulin to the latter effect, have been principal foci of our interest. Highly purified growth hormone, assayed for residual impurities, has been made available to us by the Endocrine Study Section, National Institutes of Health. With this

![Graph](image-url)
preparation, we have verified our early finding that in depancreatized dogs receiving a constant amount of food and insulin, nitrogen storage is not induced by growth hormone but is induced if the insulin dose is increased when growth hormone is given. We also found the purified growth hormone, which is virtually free of corticotropin, as diabetogenic as earlier crude preparations in the depancreatized dog. Since diabetogenic effects of crude preparations were mild in adrenalectomized depancreatized dogs, there is further work ahead.

Morphologic and physiologic effects that endocrine glands exert upon one another, as well as synergisms between products of these glands, create a rather complicated situation. Nowhere is this better illustrated than in the stubborn problem of calorigenesis, recently attacked with renewed vigor by Evans, Contopoulos, and Simpson, and Evans, Simpson, and Evans. Transferring from the multiorgan level to the subcellular ribonucleoprotein particle, one finds interesting studies on protein synthesis and on effects of growth hormone by Balis et al.. The pituitary will continue to provide problems for many scientists for some time.

At our last previous scientific meeting, in Washington, D.C., I presented some studies on effects of growth hormone and corticotropin on metabolism of N\(^15\) from glycine, alanine, and ammonium citrate. I will close with another study that has not been discussed with this group. Studies of Bartlett and Glynn, Beaton et al., and Zuchlewski and Gaebler indicated that growth hormone alters the activity of glutamic oxalacetic transaminase in muscle, and particularly the glutamic pyruvic transaminase in liver (see Fig. 4, Ref. 37). The profound reduction in activity of hepatic GPT naturally aroused our interest in knowing whether the nitrogen transfer pattern is altered.

In recent studies by Lees and Gaebler, each of four groups of hypophysectomized rats received, by intraperitoneal injection, one of the four N\(^15\)-labeled amino acids—glycine, L-alanine, L-aspartic acid, and L-glutamic acid. The groups were divided into untreated control and growth-hormone-treated subgroups. Forty-eight hours after injection of the labeled amino acids, the animals were sacrificed. Proteins from the myofibrillar fraction of muscle were hydrolyzed, and 7 amino acids, as well as ammonia, were isolated by column chromatography. Mass spectrometer determinations of N\(^15\) excess in these compounds yielded several results of interest. The amino acids from control groups, with the exception of threonine, were labeled. Alanine, glutamic acid, and aspartic acid, as well as amide nitrogen received considerable amounts of N\(^15\). When N\(^15\)-glycine was given, serine was heavily labeled. These findings are well known in the case of normal rats; the point of interest is that nitrogen transfer was not essentially altered in the absence of pituitary hormones. Growth hormone greatly increased the extent of incorporation of N\(^15\) into the amino acids and amide nitrogen of myofibrillar protein, but did not alter the nitrogen transfer pattern.

While browsing in a book containing aphorisms of Sir William Osler, I came upon the following: “One can get along with a few ounces of kidney and less liver. The liver is comparatively harmless and useless. It was put in merely for packing purposes.” No doubt, the last two quoted sentences were only meant to emphasize the first one. I do not mean to imply that determination of tissue enzyme activities is
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a useless pursuit, on which my associates and many other investigators have wasted a lot of time, but I do feel that if one can get along with so little liver or kidney, the significance of even large changes in tissue enzyme activities must be considered with caution. Whenever an experimental approach is possible, experiments with the substrates in question should be carried out.

REFERENCES