A century of animal clinical biochemistry: growth, maturity and visions for the future

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ABSTRACT

Animal clinical biochemistry can trace its origins to the physicians of ancient times gazing at their "harrglasses", presumably after they had tasted the urine for sweetness as evidence of diabetes mellitus. From these humble and rather heroic beginings, clinical biochemistry has evolved into a key diagnostic focus in human and veterinary medicine, animal science, nutrition, public health and food safety, environmental health and safety, pharmaceutical development and safety, health surveys from prenatal to birth to death and yes, even to our psychological well-being. The breadth and depth of this focus on health and disease is no less remarkable for its having evolved to its present status only within the last century. Prior to the last century, it was the growth of chemistry in the 19th century which predictably expanded chemistry into medicine as a means for identifying the compounds in body fluids, mainly the urine, that were associated with certain diseases. It soon followed that links were made as to how these chemical compounds in these fluids might have been made converted into harmful substances. Thus, the emphasis on animals focused on anabolism and catabolism of compounds and the general concept of metabolism was born. The science of animal biochemistry developed with remarkable rapidity into its present state where biochemistry is the fundamental basis for the understanding of all aspects of animal biology. In this regard, new knowledge is continually being generated and new aberrations or diseases are constantly being uncovered with ever increasing rapidity. This also means that the animal clinical biochemist must be at the forefront of all aspects of biology in order to continue to make meaningful contributions to the field. In this way, the field will continue to expand for the benefit of human and animal life in it's broadest context.

KEY-WORDS: animal biochemistry - clinical biochemistry - veterinary biochemistry.

RÉSUMÉ

Un siècle de biochimie clinique animale : développement, maturité et perspectives pour le futur. Par J. J. KANEKO.

La biochimie clinique peut faire remonter ses origines aux médecins d'autrefois qui "miraient les urines", probablement après les avoir goûtées pour détecter le goût sucré révélateur du diabète sucré. De ces débuts humbles et plutôt héroïques, la biochimie a évolué pour devenir un outil diagnostique essentiel en médecine humaine et vétérinaire, élevage, nutrition, santé publique et sécurité alimentaire, développement de médicaments, médecine préventive depuis le fœtus jusqu'à la mort et, même notre bien-être psychologique. La profondeur et l'évolution de cet intérêt porté à la santé et à la maladie est d'autant plus remarquable qu'il a évolué vers son état actuel au cours du seul dernier siècle. Auparavant, c'est le développement de la chimie au XIXe siècle qui a fait pénétrer la chimie dans la médecine en identifiant les composés des fluides de l'organisme, principalement de l'urine, qui étaient associés à certaines affections. Il s'en suit que des liens ont été établis pour déterminer comment ces composés pouvaient avoir été transformés en substances nocives. Ainsi, chez les animaux, l'accent a-t-il porté sur l'anabolisme et le catabolisme de composés et le concept général de métabolisme est alors né. La science de la biochimie clinique animale s'est développée avec une rapidité remarquable jusqu'à son stade actuel où la biochimie est la base fondamentale pour la compréhension de tous les aspects de la biologie animale. A cet égard, de nouvelles connaissances sont constamment produites et de nouvelles anomalies ou maladies sont constamment découvertes avec une rapidité croissante. Cela signifie également que le spécialiste de biochimie animale doit être au premier plan de tous les aspects de la biologie pour continuer à apporter une contribution significative à ce champ disciplinaire. Ainsi, celui-ci continuera à se développer au bénéfice de la vie animale et humaine dans son sens le plus large.

MOTS-CLÉS: biochimie animale - biochimie clinique - biochimie vétérinaire.
Introduction

Animal clinical biochemistry is particularly well characterized by the pictures of the physicians of old gaz ing at their "harglases", presumably after they had tasted the urine for sweetness as evidence of diabetes mellitus. From these humble and rather heroic beginnings, the science of clinical biochemistry has evolved into a key diagnostic focus in human and veterinary medicine, animal science, nutrition, public health and food safety, environmental health and safety, pharmaceutical development and safety, health surveys from prenatal to birth to death and yes, even to our psychological well-being. The breadth and depth of this focus on health and disease is no less remarkable for its having evolved to its present status only within the last century. Prior to this period, the growth of chemistry in the 19th century predictably expanded into medicine as a means for identifying the compounds in body fluids that were associated with certain diseases. It followed that links were made as to how these chemical compounds in these fluids might have been made in excess or not further converted into harmless substances. Thus, the emphasis on animals focused on anabolism and catabolism of compounds and the general concept of metabolism was born. The science of animal biochemistry developed with remarkable rapidity into its present state where biochemistry is the fundamental basis for the understanding of all aspects of animal biology. In this regard, new knowledge is continually being generated and new aberrations or diseases are constantly being uncovered with ever increasing rapidity. This also means that the animal clinical biochemist must be at the forefront in generating and appraising the implications to medicine in order to continue to make meaningful contributions to the field.

Simultaneously as we review these important aspects of advances in clinical biochemistry, we must recognize that these have resulted from studies of a wide variety of animal species; the mouse, rat, rabbit, cat, dog and non-human primate all have contributed and continue to contribute to our expanding knowledge. In keeping with the importance of the contribution of animals, the field will continue to expand for the benefit of human as well as animal life in its broadest context.

History

While the urine "taste test" might be regarded as the fore-runner of clinical diagnostic tests to be applied in the field of medicine, this was a physical test and it required about two millenia before a chemical test was developed for clinical use. Shortly after the identification of sugars by crystallization of their osazones, the method was applied to urine and confirmed that indeed the sweetness of diabetic urines was due to glucose. This was a true chemical diagnostic test which could be applied to a body fluid and used to confirm the presence of a specific disease entity. As the science of chemistry progressed and as new chemical compounds were identified in nature, these same compounds were sought first in urine as an easily obtained body fluid and then very quickly in blood and other fluids. While the original methods were painstaking and laborious, concerted efforts of the mid-20th century resulted in the rapid and multiple chemical analyses which are commonplace today.

In the early 1900s, clinical biochemistry was focused upon the chemical analysis of urine in keeping with the early recognition of urinary glucose and its importance in the diagnosis of diabetes. In one veterinary text of the 40s entitled "Diagnostic Methods in Veterinary Medicine", [1], for example, a book of 389 pages, a chapter entitled "Clinical Biochemistry" encompassed a mere 12 pages, 8 of which were devoted to describing urine tests for specific gravity, pH, albumin, bile, bile salts, hemoglobin, sugar, indican, ketones, glucuronic acids, and urea. Only 4 pages were used to describe blood analyses for glucose, urea, calcium, magnesium and bilirubin. In contrast, the 5th edition of "Clinical Biochemistry of Domestic Animals" [7], a book devoted to animal clinical biochemistry, consists of 932 pages with 30 chapters contributed by 35 authors. The growth of the field has indeed been remarkable.

Growth

The growth of clinical biochemistry into its various subclasses parallels the growth of biochemistry into the fields of carbohydrate, lipid and protein biochemistry and how they relate to the function of the various organ systems. Only a few of the many advances are described here as examples of the growth of animal clinical biochemistry. The classic work of BANTING and BEST in 1921 in obtaining an extract from a dog's pancreas was quickly followed by the demonstration that this extract could be used to treat the diabetes of a depancreatized dog. This extract was named insulin for its source, the Islets of Langerhans. Very quickly, the extract was used successfully to treat human diabetic patients. All this created the excitement that a cure for this disease which had afflicted humankind since time immemorial was now in the offing. This also created the scientific excitement that translated into the general feeling that all medical problems were solvable through research in biochemistry. The discovery of insulin provided the impetus for the rapid growth in all fields of carbohydrate metabolism and diseases associated with its failure. This same impetus translated into an equally rapid growth in all phases of biochemistry as it related to medicine and continues to the present day. For example, in this period, protein research expanded greatly and in 1959, the structure of insulin [11] was finally established. A sobering thought, that in spite of all efforts, diabetes has increased to the point where it is now the 3d leading cause of death in the U.S.

The growth of clinical enzymology is another good example of the growth of a subclass of biochemistry into its present position of fundamental importance in the clinical diagnostic laboratory today. In the late 1800s, the enzyme urease had been crystallized and by 1900, only a few digestive enzymes were known. These were trypsin (proteinase), pepsin (proteinase), diastase (amylase) and ptyalin (salivary amylase). Amylases had been detected in urine but their diagnostic implications were unknown. In the 1920s, alkaline phosphatases in blood were recognized to be useful adjuncts for the diagnosis of bone and liver disease. In the 1930s, acid
phosphatase was used as a diagnostic test for prostatic cancer, a forerunner of modern tumor markers. In the early 1950s, the enzyme glutamic oxaloacetic transaminase (SGOT) was known in blood [8] but it was shown in 1955 to be elevated in the blood of our President Eisenhower during his heart attack. From this initial finding, the spectacular growth of the field of clinical enzymology began to reach its position of prominence today.

**Maturity**

By the 1950s, the field of clinical biochemistry had grown sufficiently to stimulate the formation of the American Association for Clinical Chemistry (AACC). From its modest beginnings, the society has now grown to become the major scientific organization in the field in the world with a membership of about 12000. This year's 52nd annual meeting of the AACC is expected to draw 20,000 participants. What are some of the factors responsible for this remarkable growth? To begin, the advances engendered by the discovery of insulin and its structure led to the general feeling that diagnosis and treatment of diseases of humans and animals could be solved by understanding the fundamental biochemical mechanisms of the disease processes. The involvement of animals was also seen as an integral part of the process, primarily as experimental subjects, although today, animals are seen as direct beneficiaries since they, too, develop diseases similar to humans. Dr. BANTING chose the dog because he knew that dogs developed diabetes when their pancreases were removed. Today, the clinical veterinarian is well aware that diabetes is an important disease which affects all animals and the dog and cat in particular.

For the animal clinical biochemist, there is the constant challenge to keep abreast of new and fundamental advances in general fields of science and more importantly, to relate these advances to human and animal diseases. As we enter the new millenium, it becomes doubly important that we appreciate the universality of science and as animal scientists, that all studies of animals have impacts upon human and animal well-being.

In this past half century, virtually all areas of research endeavor human and animal well-being have reached a sophistication undreamed of during the prior centuries. In 1908, Sir Archibald GARROD first developed the concept of "Inborn Errors of Metabolism" in his Croonian Lectures presented to the Royal College of Physicians. He described the similarities between albinism, alkaptonuria, cystinuria and pentosuria. He noted that in these 4 conditions, they occurred very early in life, many had a familial occurrence and many were associated with consanguineous marriages. From these early observations, the concept has been established and now more than 300 hereditary diseases in humans are recognized. Similarly, numerous hereditary diseases are now recognized in animals and are generally counterparts of the same or similar diseases of humans. An early finding of a hereditary disease in animals was the discovery of "pink tooth" in South Africa in 1936 by FOURIE [4] and RIMINGTON [9]. It is now known that "pink tooth" is Congenital Erythropoietic Porphyria (CEP), a disease caused by the hereditary deficiency of the heme synthetic enzyme, Uroporphrinogen III cosynthetase in humans or in animals.

The development of liver function tests offers another example of diagnostic maturity for a disease which has historically plagued humankind. The appearance of "jaundice" or "icterus" has long been associated with liver disease. Bilirubin was found in bovine gallstones and associated with hemoglobin in the late 1800s [15]. It was not until 1916 that VAN DEN BERGH and MULLER [14] established that there were two forms of this yellow pigment in the blood of some human cases of icterus. One form produced a violet color immediately when sulfanilic acid was added to the serum. When alcohol was then added to the mixture, a further increase in the violet color occurred. They designated the first form as "direct reacting" bilirubin and the form which appeared after the addition of alcohol as "indirect reacting" bilirubin. These terms are with us to this day. In 1953, COLE and LATHE [2] using reverse phase chromatography were finally able to separate these two chemical forms of bilirubins which are now known as the unconjugated or free form of bilirubin and the glucuronic acid conjugated or bound form. Bilirubin fractionations have since been a virtual mainstay of liver diagnostics in animal clinical biochemistry.

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Virtual parallel to bilirubin, clinical enzymology in animals developed as an important tool in liver diagnostics and is widely used. The finding of the usefulness of AST in cardiac disease of humans along with the almost simultaneous finding that AST was elevated in liver disease provided the impetus for the growth in clinical enzymology. A myriad of serum enzymes have been investigated in humans and in animals to identify serum enzymes with potential usefulness as diagnostic tools in virtually all diseases. Few have withstood the test of time and today a handful of clinically useful diagnostic enzyme are in widespread use in liver diagnostics.

A third clinically useful class of biochemical as relates to liver diagnostics is the bile acid. Bile acids form a large group of compounds found in the bile, are important in the intestinal digestive process and have become extremely useful diagnostic aids in liver disease. Most of the work with bile acids was performed in the last quarter century and rather quickly, the determination of the total bile acids has become an important diagnostic tool. The enterohepatic circulation of the bile acids parallels that of the bilirubins but the presence of 12 - 18 different types of acids has made their fractionation a daunting challenge which has not been resolved to this day. Nonetheless, the total bile acids have become very useful in the diagnostic armamentaria in veterinary diagnostics.

Technologically significant advances have had equally significant impacts upon clinical laboratory science as have the chemical advances. In the first half of the 20th century, all chemical laboratory analyses were by manual methods, labor intensive, time consuming and therefore relatively expensive. The long turn around times also meant that there was little incentive for wide usage of any test even if it was available. The list of these manual tests is long and some commonly used tests of the time are: BENEDICT's test for glucose, biuret test for proteins, cephalin-cholesterol flocculation test for hyperglobulinemia or hypoalbuminemia, VAN
DEN BERGH'S test for bilirubin, etc. In the 1960’s, an automated mechanical system was invented which could analyze a single serum sample for several different analytes, including glucose, urea nitrogen, albumin, total protein and some enzymes. After many failed attempts to find a taker, the Technicon Corporation in Tarrytown undertook to manufacture and market the pioneer Sequential Multiple Analyzer, the SMA 12 which could analyze a small serum sample for 12 different analytes. From these humble beginnings, automated, computerized analyzers are now commonplace and have become the heart of the clinical biochemical laboratory. Modern automated analyzers are now programmable, selective and have the capabilities to report their data to remote computer stations. Many have menus of tests of upwards of 35 different tests and more are constantly coming on line.

Amongst the signal advances in animal clinical biochemistry was the development the immunoassay technique for the assay of insulin [16]. The immunobinding technique had the great advantage of high accuracy in the presence of low levels of hormone. Quickly, the concept of competitive hormone binding to binding proteins was applied to all protein hormones for which specific antibodies as binding proteins could be produced. These assays for virtually all hormones are commonplace in the clinical laboratory today.

All of these advances have moved laboratory science into the 21st century with a formidable array of capabilities. Even more so, these advances have contributed a staggering amount of information relative to the fundamental understanding of the disease process in animals. One need only to peruse the table of contents of the 5th edition of "Clinical Biochemistry of Domestic Animals" [7] to see the scope of animal clinical biochemistry today. Even though this table of contents is long, new information in additional areas is constantly forthcoming and indicates that the future for animal clinical biochemistry is bright. I firmly believe that future editions or books of this type will bear out the truism of this observation.

**Future**

First of all, DNA technology with all its ramifications are only just recently being exploited in animal clinical biochemistry although DNA probes have been used for several decades in the microbiological laboratory for the identification of various pathogens. In animal clinical biochemistry, understanding of its genome would be of particular value in uncovering and diagnosis of hereditary diseases. Mutations, deletions and insertions are among the many different ways that the production of a given gene product could be affected.

A number of examples have been reviewed by HAUVE [6] and they include citrullinemia in Australian Friesian cattle, malignant hyperthermia (MH) in pigs, leukocyte adhesion deficiency (LAD) in cattle, hyperkalemic periodic paralysis (HYPP) in quarter horses, uridine monophosphate synthase deficiency (DUMPS) in Holstein cattle and maple syrup urine disease of Australian Polled Hereford cattle. Another group are the lysosomal storage diseases (LSD) [5]. The LSD are a rare group of enzyme deficiencies involved in cell catabolism. The enzyme deficiencies are expressed in the accumulation of their substrates within the lysosomes, hence the name LSD and which may at times be visible even under the light microscope. These substrate accumulations may result in the increases in cell, tissue and organ sizes which are fundamental characteristics of the LSD and which in turn disrupt their function. While the enzyme deficiency may be detected by assay, the best diagnosis is achieved by analysis of the coding sequence for the deficient enzyme.

Another important facet which has yet to be pursued to its fullest by the animal clinical biochemist is the burgeoning field of tumor markers. As the longevity of our domestic animal population increases, tumors of all types can be expected to increase in frequency and with it needs for early and accurate diagnosis. While concerted efforts and progress have been made in human medicine, much can be done in veterinary medicine and animal science. Biochemical markers of tumors have been of great interest in the medical community and the more so with the success of the prostate specific antigen (PSA) assay in identifying human prostate cancers. Identification of these markers remains a formidable task but simultaneously offer great opportunities for contributions by animal clinical biochemists. Thyroglobulins (Tg) are used as markers of thyroid carcinoma and are being used in thyroid screens in conjunction could with the thyroperoxidase antibody (TPOAb), an index of autoimmunity. Currently, TSH assays of sufficient sensitivity that the American Thyroid Association recommends a TSH based, cost effective strategy for thyroid disease diagnostics [12] and for initial screening. These assays for TSH have a functional sensitivity of 0.01 - 0.02 mIU/l [10] which is entirely sufficient for hypothyroidism and in most cases for hyperthyroids. Now, a TSH assay specifically for the dog [13] is commercially available. This was made possible by the preparation of a highly purified canine TSH from which a monoclonal and a polyclonal TSH antibody were raised. TSH reference range in 38 dogs was 0.03 - 0.27 ng/ml (0.10 ng/ml). In two thyroidectomized dogs, the TSH was 2.7 and 2.8 ng/ml. In 4 radiothyroidectomized dogs, the mean TSH was 4.3 ng/ml. It would be of great interest to extend these studies to the hypothyroid horse and to the hyperthyroid cat.

In humans, about 90 % of all hypothyroids are thought to be the result of autoimmunity. The presently most effective assay for this autoimmunity is the thyroperoxidase autoantibody (TPOAb). The test is positive in Hashimoto's thyroiditis, primary thyroiditis, thyroiditis, post-partum thyroiditis and neonatal thyroiditis. It is negative in thyroid carcinomas and in hyperthyroidism (Grave's disease) [3]. These are difficult assays but are potentially useful in differentiating carcinomas from hyperplasias in the hyperthyroid cat.

In more traditional areas of animal clinical biochemistry, especially among our "geriatric" companion animals, we must continue to focus on new and better ways to diagnose and manage their diseases. Diabetes continues to be a challenge for all animal clinical biochemists in all facets of its diagnosis, management and care. New ways of evaluating hyperglycemias and of monitoring the success of therapy through the use of glycated hemoglobins (HbA1c) and fruc-

tosamine (FrAm) have improved patient care but nowhere near it’s potential. Additionally, assays for insulin, proinsulin, C-peptide and insulin antibody need to be brought into the clinical arena.

New approaches beyond the traditional delivery of laboratory services are now coming to the forefront with the development of sophisticated devices for “Point-of-Care Testing” (POCT). These systems are the fruition of many earlier attempts to simplify and make available laboratory results at the bedside rather than in the large central laboratory. Only recently with the aid of miniaturization and computerization has POCT become widely used, particularly in the intensive care unit. The US market is now upwards of 4 billion SUS and growing. POCT instruments are capable of calculating parameters as well as interfacing with central laboratory computers to take full advantage of all the data support that the central laboratory has to offer. POCT instruments are particularly well suited for the field or mobile veterinary unit.

In the final analysis, the animal clinical biochemist must be positioned as a scientist with a unique capability to cross disciplinary and species lines. The need to understand interspecies differences is in marked contrast to the single species focus of human medicine. This also means that the animal clinical biochemist must keep abreast of new developments in the broad field of animal biology and must also be a generator of new knowledge in the field. By definition, this means that research and dissemination of new knowledge is an inherent responsibility of the animal clinical biochemist. This is the only way in which the animal clinical biochemist can fully participate as a provider/colleague/collaborator in the traditional delivery of animal health care, on the research team or as an independent scientist/principal investigator in the range of studies involving animals. This implies that the animal clinical biochemist must broaden his/her knowledge of clinical medicine in order to converse freely in the ”language of medicine”. The definition of the animal clinical biochemist as a biochemist with clinical expertise means that the animal clinical biochemist must be knowledgeable in one or more aspects of the disciplines basic to medicine: anatomy, biochemistry, physiology, cell biology, molecular biology, pathology, immunology, nutrition or their subdisciplines. The IFCC further defines clinical chemistry as ”the application of chemical, molecular and cell concepts and techniques to the understanding and evaluation of human health and disease”, a clear call for involvement in medical outcomes.

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