

# A mouse is not a man is not a dog 2000 or species specificity in clinical chemistry

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## SUMMARY

This paper reviews the types of biochemical differences among species, emphasizing and presenting numerous examples of species specific differences in serum enzymes and in hormones. Knowledge of species specific differences in clinical biochemistry and physiology enable the investigator to select an appropriate animal surrogate for man, and provide optimal interpretation for clinical laboratory findings.

**KEY-WORDS :** species-specificity - laboratory animals - transgenic animals - enzymes - hormones.

The animal clinical biochemist has a unique contribution to offer in biomedical research studies in that he or she has sufficient knowledge of human clinical biochemistry to be able to evaluate the role of species differences when an animal species is used as a surrogate for man. The development of transgenic animals has resulted in numerous models which generally resemble the background species, but in some distinct respects, appear to be the graft species. In this presentation I will present a review of some differences and, conversely, similarities among species, with a few examples of transgenic animals, as an aid in selecting appropriate surrogates for man.

The scientist working in human clinical biochemistry is generally aware of the fact that reference values may differ by species, but may not know that species may differ in five other important respects : 1) The analyte may differ by species. In man, dog, and nonhuman primate, the principal glucocorticoid is cortisol ; in the mouse and rat, it is corticosterone. The hamster and the rabbit have significant levels of

## RÉSUMÉ

**Une souris n'est pas un homme ni un chien 2000, ou la spécificité d'espèce en biochimie clinique. Par W.F. LOEB.**

Cet article passe en revue les types de différences biochimiques interspécifiques, en mettant l'accent sur la présentation de nombreux exemples de différences interspécifiques pour les enzymes et les hormones sériques. La connaissance des différences interspécifiques en chimie clinique et en physiologie permet au chercheur de sélectionner un substitut adéquat pour l'homme et de fournir une interprétation optimale des données du laboratoire de biologie.

**MOTS-CLÉS :** spécificité d'espèce - animaux transgéniques - enzymes - hormones - animaux de laboratoire.

both cortisol and corticosterone ; in the hamster the predominant one varies with a circadian rhythm [6]. 2) The analyte may have a different tissue of origin. In man and rat, salivary amylase contributes to the total serum amylase, and sialoadenitis may elevate the serum amylase. In the dog and rabbit, the salivary glands have only minuscule amounts of amylase activity, which does not contribute significantly to serum amylase, even in sialoadenitis. Similarly, the predominant intracellular origin of an analyte may differ by species. In man, nonhuman primate, dog, and rat, the principal source of alanine aminotransferase is cytoplasmic, while in the guinea pig it is mitochondrial [2, 4]. 3) The turnover time of the analyte may vary by species. In the dog, the half life of the liver isoenzyme of alkaline phosphatase is approximately three days while in the cat it is approximately six hours. This longer half life in the dog makes the enzyme a more sensitive marker than in the cat [4, 7]. 4) The clearance mechanism may differ by species. In man and in the mouse, pancreatic amylase is cleared through the kidney ; hyperamylasemia is

followed by amylasuria. By contrast, in the dog, less than 1 % of pancreatic amylase in serum is cleared through the kidney, so that hyperamylasemia of pancreatic origin does not result in amylasuria [4]. 5) Different analytic methods may be required depending on the species. This is especially important with respect to immunoassays for protein and peptide substances which may have differing amino acid sequences by species, so that the antibody fails to recognize the antigen completely or at all. This will be discussed extensively later in this presentation. The measurement of albumin by the bromocresol green method, using human or bovine standard is quite satisfactory for most species including dog, rat, and mouse. However the binding of bromocresol green to rabbit albumin is much greater, and overestimates the concentration of albumin in the rabbit by approximately 50 % unless rabbit albumin is used as the standard. Similarly, the absorbance of fibrin monomere at 405 nm differs by species, being highest in man, lowest in the rabbit, and intermediate in dog, rat, and pig. The accurate measurement of fibrin monomere for each of these species requires a species-specific calibration curve.

For each species, the effects of sex, age, strain or breed, response to stress including restraint, anesthetic agents, and other variables unrelated to the specific experiment must be known. Control animals should be treated identically to test animals except for the specific test article or procedure. In oral dosing studies in which the vehicle is other than isotonic saline or water, it is advantageous to have two control groups, one dosed with water or saline only, the other dosed with the vehicle. The vehicle alone may induce clinical chemical changes. This can only be recognized if there are both saline or water and vehicle control groups.

In any instance, the controls must be dosed like the test animals to subject them to the same stress of handling.

Different species respond differently to stress. Some years ago we developed an intravenous glucose tolerance test for nonhuman primates. We compared the effect of manual restraint, chemorestraint using ketamine, and anesthesia using pentobarbital in male and female rhesus monkeys (*Macaca mulatta*) and African green monkeys (*Cercopithecus aethiops*). Each animal was tested using each method of restraint. Summarizing our findings, results were similar in animals chemorestrained with ketamine or anesthetized with pentobarbital. Manually restrained male and female African green monkeys and male rhesus monkeys had significantly different results due to stress, results which would be misinterpreted to infer that the animal was diabetic. Female rhesus monkeys, which are somewhat more docile than males or African green monkeys of either sex did not have significantly different results when manually restrained than when chemorestrained or anesthetized [3]. An interesting study of the effect of stress in pigs was conducted by loading the pigs onto a truck, then unloading them again. Clearly this is quite a mild stressor. In addition to the increases in cortisol and glucose which might be anticipated, there were transient increases in insulin, lactate, thyroxine, and triiodothyronine, and decreases followed by increases in somatomedin and free fatty acids [11].

The measurement of so-called "leakage enzymes" has given us a powerful tool for recognizing injury to various tissues and organs, particularly the liver. The sensitivity and specificity of a particular enzyme in a particular species depends on the tissue and cellular location and the rate of clearance or half-life [2]. Alanine aminotransferase consists of two isoenzymes, one present in the cytosol, the other in the mitochondria. In cellular injury, the cytosol isoenzyme is released much more readily than the mitochondrial one. In the rat, the liver has a much higher activity of ALT per gram than any other tissue. The cytosol isoenzyme is the predominant one. Consequently in the rat, ALT is a sensitive and specific marker of hepatocellular cell membrane injury, as it is, to a greater or lesser degree in the mouse, dog, nonhuman primate, and man. By contrast, in the guinea pig, the activity of ALT per gram of liver and heart is comparable, and predominant isoenzyme is the mitochondrial one. As release of the mitochondrial isoenzyme into the serum requires necrosis of the individual cell, ALT is neither sensitive nor specific as a marker of hepatocellular injury in the guinea pig [2, 4]. Sorbitol dehydrogenase, a cytoplasmic enzyme, is a valuable sensitive and specific marker of hepatocellular injury in all laboratory mammals as its distribution is predominantly hepatic [2, 4]. The alkaline phosphatases, enzymes which hydrolyze monophosphate esters at an alkaline pH, are induction enzymes rather than leakage enzymes. The isoenzymes are controlled by three genes in man and great apes, two genes in other mammals. Isoforms are the result of post translation glycosylation. The dog has a unique alkaline phosphatase isoenzyme, associated with endogenous or exogenous increase in glucocorticoids. This corticosteroid-induced alkaline phosphatase appears to be produced by the liver, specifically in response to glucocorticoid stimulation. In dogs with Cushing's disease or on glucocorticoid therapy, this may be the predominant isoenzyme. The other isoenzymes making a major contribution to the serum alkaline phosphatase activity in the dog are the hepatic (biliary) form and the bone isoenzyme. The isoenzymes predominating in the serum of the dog have a half life approximating three days. By contrast, the intestinal, renal, and placental isoenzymes of alkaline phosphatase in canine serum have half-lives approximating 6 minutes. Consequently, these isoenzymes do not make a significant contribution to the total serum alkaline phosphatase activity. The clearance of intestinal alkaline phosphatase in the rat is biphasic, with a first phase of 2.8 minutes and a second phase of approximately 60 minutes. In the rat, the intestinal isoenzyme of alkaline phosphatase is present in the highest activity in the serum, followed by the hepatobiliary and bone isoenzymes. Feeding rats a high fat meal elevates serum alkaline phosphatase, while depriving them of food decreases total serum alkaline phosphatase via a decrease in the intestinal isoenzyme. This demonstrates the importance of bleeding animals in a study in a randomized fashion. If rats are deprived of food and are bled in a treatment group related sequence, controls first, followed by low, then intermediate, then high dose, the high dose animals may possibly have significantly lower alkaline phosphatase activity, not as a result of treatment, but of the bleeding sequence [4]. In the cat, the hepatobiliary isoen-

zyme of alkaline phosphatase has a half-life approximating 6 hours in contrast with the half-life of three days in the dog [7]. The more rapid excretion in the cat explains the lower reference values and lower sensitivity for cholestasis in the cat than in the dog.

In all species which have been studied, values for total alkaline phosphatase are higher in infant and juvenile animals than in adults, the result of higher bone alkaline phosphatase during bone growth. Values decrease after the neonatal period, corresponding to the decrease in bone growth rate.

Species specificity in an enzyme-substrate reaction was demonstrated in a study of transgenic mice. Human renin reacts specifically with human angiotensinogen to release angiotensin I, converted by angiotensin converting enzyme (ACE) to angiotensin II. Rat or mouse angiotensinogen is very resistant to the action of human renin. Transgenic mice which express human angiotensinogen and other transgenic mice which express human renin have been produced. Both of these types of transgenic mice are normotensive. Crossbreeding of these transgenic mice results in mice which express both human angiotensinogen and human renin. These so-called dual gene strain mice are hypertensive, but respond to renin inhibitors, ACE inhibitors, and angiotensin II receptor antagonists. Similar studies have been performed in rats [13].

Next, I want to consider some examples of species specificity in hormones. The insulin molecules of man, nonhuman primates, dogs, swine, cattle and rabbits are immunologically quite similar, and can be accurately measured by the human insulin immunoassay. Rats, mice, and hamsters have two different insulin molecules termed Insulin I and Insulin II. In immunoassays they cross react poorly with the insulin of man, but are quite similar among the three rodent species and cross react well among these species. As both of these molecules have similar function, the antibody used to assay rat, mouse, and hamster insulin must recognize Insulin I and Insulin II equally [10]. The guinea pig has yet a different structure, and cannot be assayed by either the immunoassay for human insulin nor the immunoassay for rat, mouse, and hamster insulin. Incidentally, while the immunoassay for human glucagon cross reacts well with the glucagons of many mammalian species including rat, mouse, and hamster, it does not recognize guinea pig glucagon [5]. To the best of my knowledge, valid assays for guinea pig insulin and glucagon are not commercially available. Insight into gluco-regulation was obtained from a transgenic mouse model constructed to produce human insulin. These mice produce mouse insulin I, mouse insulin II, and human insulin. Their serum glucose, total serum insulin levels, and responses to challenges to gluco-regulation are comparable to those of conventional mice. This demonstrates the importance of post-transcriptional controls in glucose and insulin regulation [9].

The immunoassays for measuring pituitary hormones, except ACTH, are quite species specific. The assay for measuring human ACTH is valid in all mammalian species in which it has been studied, including all laboratory and domestic mammals. The antibody is raised to the first 24 amino acids, which appear to be similar in all mammals,

including rodents in which the ACTH molecule, resulting from cleavage of proopiomelanocortin, is approximately twice as large as in the remaining species [6]. For immunoassays for pituitary hormones other than ACTH, species specific reagents should be used. The pituitary gonadotropins, FSH and LH, chorionic gonadotropin in the species in which it occurs, and TSH are glycoprotein hormones consisting of an  $\alpha$  and a  $\beta$  subunit. The  $\alpha$  subunit is highly species specific and is similar among the hormones of this class within a given species, that is, it confers the species specificity to the hormone. The  $\beta$  subunit conveys the function to the molecule, and the  $\beta$  subunits for a given hormone are similar, though not identical among various species.

Growth hormone is the prototype of a substance released with an ultradian rhythm, in approximately seven pulses a day. Serum levels undergo large swings, beyond the control of the investigator. Therefore, small numbers of measurements, or measurements on a small number of animals are essentially meaningless. Only mean values on a large number of animals are meaningful. This phenomenon occurs to a lesser degree with respect to luteinizing hormone and testosterone, both of which are released in a pulsatile manner [6]. Studies on transgenic mice have shown that the growth hormones of different species differ in function as well as structure. Transgenic mice expressing human growth hormone, rat growth hormone, and bovine growth hormone have been developed [1, 12]. Transgenic mice expressing both mouse and human growth hormone appear normal at birth. At about four weeks of age, growth becomes accelerated due to the stimulation of insulin-like growth factor 1 (Somatomedin C) by growth hormone. Males express approximately twice the concentration of human growth hormone as females. Adult transgenic mice expressing human growth hormone weigh 50-100 % more than conventional mice of the background strain. The transgenic mice age faster and have shorter lifespans than conventional mice. The transgenic females are sterile. Ovarian function and ovulation appear to be normal. In the mouse, mating induced prolactin release is required for the activation and maintenance of luteal function. Human growth hormone fails to support this. If female transgenic mice, producing human growth hormone, breed and are supported by progesterone injections or prolactin secreting pituitary implants, they become pregnant and deliver live pups, but generally do not lactate adequately for the survival of the pups. Male transgenic mice expressing human growth hormone are fertile but have a shortened reproductive lifespan, and decreased reproductive aggressiveness with age [1].

Although the structure of thyroxine (T4) and of triiodothyronine (T3) are identical in all mammalian species, there are major differences in the structure, electrophoretic migration, and binding affinity of thyroid hormone transport proteins. Many species of interest to the animal clinical biochemist have lower, sometimes much lower, concentrations of total serum or plasma T4 than man. Since only the free hormone is physiologically active, this may be due to the greater binding affinity of the principal human transport protein, thyroxine binding globulin, than the transport proteins of other species. There is controversy in the literature as to whether the rat has thyroxine binding globulin, but it is agreed that it does not

play a major role as in man. In the rat, the principal thyroid hormone transport proteins are transthyretin, formerly called thyroxine binding prealbumin, and albumin. In the mouse, transthyretin binds T3 but not T4. T4 is transported bound to albumin and an  $\alpha$  globulin. Transthyretin appears to exist in all species, though its electrophoretic migration may vary somewhat among species [8].

In summary, knowledge of species specific differences in clinical biochemistry and physiology enable the investigator to select an appropriate animal surrogate for man, and provide optimal interpretation for clinical laboratory findings.

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