Pharmaceutical Research, Vol. 11, No. 2, 1994

Commentary

Further Commentary: Physiological Parameters in Laboratory Animals and Humans

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In their article presenting "acceptable" values for body weights, volumes of body fluids and organs, blood flow through organs, gastrointestinal variables, and miscellaneous physiological parameters of the mouse, rat, rabbit, monkey, dog, and human, Davies and Morris (1) give a single mean reference value for each parameter which they derived from an extensive search of the literature. While these values may be useful for broad interspecies scaling, they represent a ballpark figure without revealing the dimensions of the playing field. What time of day or year were the data collected? Are sexes combined? A great problem in this age of information is that researchers are deluged with numbers, but Davies and Morris have tried to simplify their reference numbers by stripping too much information critical to the interpretation and usefulness of the means listed. While they mention "reasonably normal ranges," they give only single mean values. The authors asked for help in completing or modifying their data base and we would like to offer several comments.

A basic requirement for reporting mean values is to list the sample size and present an appropriate indicator of measurement error or uncertainty, such as a standard error (SE), a standard deviation (SD), or a confidence interval (CI) (2). A mean value derived from 500 individuals is much more meaningful for the population than a mean derived from 50 or only 5 individuals. Since we need to know the interval of values that is likely to contain the true mean, a CI which encompasses this range of values is helpful (3,4). For the computation of reference intervals, there is an unwritten convention to compute the central 95% interval (4), which is calculated as (a) ± 1.96 SE from the mean for estimation of an interval in which the true mean may occur or (b) ± 1.96 SD from the mean for description of the data distribution about the computed mean. While consistent use of the 95% CI (± 1.96 SE or SD) is preferable, 90 or 99% can also be employed in certain instances. Use of a CI will allow the reader to assess whether an observed value is located within or without a reference interval, as opposed to above or below a single mean value, thereby making the tables of Davies and Morris much more meaningful. Sexes must also be listed separately, if possible.

Many of the changes occurring in living systems are rhythmic and thus predictable to a certain degree. Many variables have been shown to exhibit chronobiological rhythms: daily, weekly, monthly, seasonal, etc. (4,5). Indeed, a circadian time structure has been shown to characterize nearly every biological function tested thus far in a wide variety of species, resulting in the ability to specify times when values are predictably higher or lower throughout each 24 hr (predictive homeostasis). Thus, in pharmaceutical research using living organisms, *when* a procedure is done will influence its outcome (5). To select times to optimize responses to pharmacologic agents, we need to know when the organism is in a phase most favorable to respond in the desired way. Reference values are needed not only for "normal ranges," but also for *when* high and low values can be expected (in relation to the sleep–wake schedule) (6) and to what extent these values can be expected to fluctuate around the mean. Different reference data may therefore be necessary for different times.

As part of a long-term aging project, we recently reported reference values for circadian rhythms in 98 variables (6 vital signs, 16 in whole blood, 50 in serum, and 26 in urine) in clinically healthy men (7). An exhaustive previous report summarized hematologic and urinary human daily, weekly, monthly, and yearly reference values (8). Circadian rhythms in the human gastrointestinal tract (stomach, small intestine, biliary tree, and liver) have been reviewed (9), with substantial day–night differences in digestive tract enzymes and gastric motility and emptying, as opposed to the single values given for humans in Table IV of Davies and Morris. At St. Paul–Ramsey Medical Center in Minnesota, the reference ranges used for hematocrit (Hct) are 39.6–47.2% for males and 34.2–43.7% for females (10), with the upper limit of the latter range below the Hct mean of 44% reported for humans by Davies and Morris in their Table V.

Turning to animal data, we recently tested 42 hematological and biochemical baseline values collected over 5 years from dogs for seasonal (circannual) rhythms and found an effect of season in 74% of the variables tested (11). The 90% range for Hct was 36.6–54.4% for all 465 dogs, with a mean $\pm$ SE of 45.7 ± 0.3%, compared with a reference value for Hct of 42% for dogs given in Table V in the Davies and Morris Commentary. Hct was significantly higher (+3%) in female dogs compared with males. Of particular importance to transplant surgeons was our finding that serum creatinine, considered a biological constant in health, varied by 18% throughout the year, from a peak in May to a trough in November. Since serum creatinine is an important marker for

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0724-8741/94/0200-0349$07.00/0

monitoring the function of kidney transplants, and its variation is the basis on which diagnostic and therapeutic maneuvers are implemented in renal allograft recipients, the circannual variation in serum creatinine may need to be considered when assessing whether or not changes in repeat serum creatinine values are of clinical significance. An extensive review of more than 2000 articles summarized circadian rhythms in 116 murine hepatic enzymes involved with lipid, sterol, amino acid, xenobiotic, and carbohydrate metabolism and energy production (12). Circadian rhythms with similar timing but differing 24-hr averages have been described for red cell and white cell parameters in three strains of mice (13). For murine Hct, the 24-hr average ±SE was as follows: CD_2F_1 males: 53.43 ± 0.12% (n = 424), CD_2F_1 females: 53.35 ± 0.17% (n = 284); BDF_1 males: 50.20 ± 0.22% (n = 89), BDF_1 females: 48.85 ± 0.39% (n = 98); Swiss Webster females: 52.66 ± 0.19% (n = 251), all of which are above the single Hct reference value of 45% listed for mice in Table V in Davies and Morris (1).

Finally, we have also observed significant seasonal changes in body and organ weights and splenic antibody formation in B6C3F1 mice studied weekly for 1 year (14). Body weight was 7% greater, while spleen and thymus weights were 27 and 13% greater, respectively, in the winter compared with the spring. A similar report on seasonality of organ weights for C3H mice is being prepared.

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