blasts of the chorion offer the possibility of recognizing viruses which do not grow in epithelial cells, such as salivary gland virus. The fibroblasts have been maintained with infrequent medium changes for more than a month. The fact that chorion cultures contained 2 types of cell made recognition of cytopathogenic effect more difficult than with cultures of a single-cell type. After 2-3 weeks the epithelial cells were often shed leaving a relatively pure culture of fibroblasts.

Summary. 1. Chorion cell cultures of mixed epithelial and fibroblast-like cell types have been grown regularly in tissue culture. 2. Susceptibility of these cells to various viruses has been explored. Polioviruses types 1, 2, & 3; Coxsackie B1, B2, B3, B4, and B5; Coxsackie A9; adenoviruses 1, 2, 3, 4, 5, 6, 7, and 9; herpes simplex; ECHO viruses 1, 3, 4, 6, and 11; vaccinia; and human salivary gland virus have been found to induce cytopathic changes in chorion cell cultures.

1. Zitcer, E. M., Fogh, J., and Dunnebacke, T. H., *Science,* 1955, v122, 30.

2. Takemoto, K. K., and Lerner, A. M., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 179.

3. Weinstein, H. J., Alexander, C., Yoshihara, G. M., and Kirby, W. M. M., *ibid.,* 1956, v92, 535.

4. Eagle, H., *Trans. Assn. Am. Physicians,* 1955, v68, 78.

5. Rowe, W. P., Hartley, J. W., Waterman, S., Turner, H. C., and Huebner, R. J., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 418.

6. Committee on ECHO Viruses. National Foundation for Infantile Paralysis, *Science,* 1955, v122, 1187.

7. Hartley, J. W., Huebner, R. J., and Rowe, W. P., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 667.

Received February 6, 1957. P.S.E.B.M., 1957, v95.

Some Normal Laboratory Values in the Dog. (23127)

JOHN E. BAER, HAROLD M. PECK, AND SAMUEL E. MCKINNEY
(Introduced by Karl H. Beyer)
Merck Institute for Therapeutic Research, West Point, Pa.

As part of preclinical evaluation of a potential drug in our laboratories the effect of long-term administration of the agent is observed in dogs. Because it is essential to know whether changes in chemistry or hematology are developing in response to a challenge with the therapeutic agent, control data are obtained on dogs prior to initiation of the agent. A compilation of these control data is presented herein. There is in the literature little systematic information concerning clinical chemical or hematologic norms in the dog in spite of extensive use of this species in laboratory work. Albritton(1) has made a critical selection of blood values from the literature, but many of the data were based on small populations, and each component was usually measured in a different group of dogs maintained under different conditions of diet and handling. This paper presents data obtained on blood and urine components in a large number of "normal" dogs under certain specified conditions. It is to be hoped that this may permit more useful correlations than would a mere compilation of the results of various tests that are at present scattered throughout the literature.

Methods. Female mongrel dogs were selected from stock on the basis of apparent good health, were dewormed, inoculated against distemper and maintained in metabolism cages for several weeks. A 500-g portion of food (25% horse meat in commercial dog food) was made available for one hour each day, after which remaining food was removed and weighed. Water was available at all times. After the dogs had become accustomed to handling and were of constant or increasing weight, weekly chemical and hematologic values were obtained prior to the daily meal. An extra 500 ml of water was given by gavage on the day preceding chemical analysis to insure an adequate volume of urine. Blood was drawn from the jugular

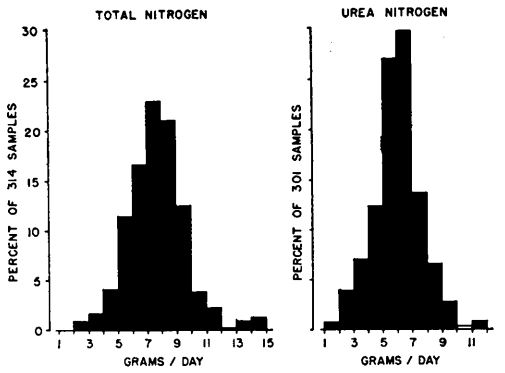

FIG. 1. Daily excretion of nitrogen by normal dogs.

vein; heparin was used as anticoagulant. When the results of 3 successive tests indicated reasonably stable values, the dog was considered to be ready for use in evaluation of a drug. The data presented below are the results of these 3 successive "triplicate control" values in over 100 dogs (total >300 determinations), except in the case of Table I. *Total and non-protein nitrogen* were determined by the method of Koch and McMeekin. *Protein* was calculated from the above as N x 6.24. *Urea* was converted to ammonia with urease and color developed with Nessler's reagent. *Creatinine* was determined with alkaline picrate. *Glucose* was determined on a tungstate filtrate with Benedict's reagent. *Total bilirubin* was measured by the method of Malloy and Evelyn, *cholesterol* by the procedure of Abell, *alkaline phosphatase* using β-glycerophosphate substrate prepared according to Shinowara, Jones and Reinhart, *phosphate* by the method of Fiske and Subba-Row, *potassium and sodium* using a Baird flame photometer with internal lithium standard, and *thymol turbidity* according to Maclagan. Literature references for these procedures may be found in Hawk, Oser and Summerson(2). Standard hematologic staining and counting technics were used. Hematocrit and sedimentation rates were measured using the Westergren method. Hemoglobin was determined by the method of Evelyn(3) adapted to the Coleman Junior Spectrophotometer.

Results. In a normal distribution, 67% of a population will fall within one standard deviation, and 95% will fall within 2 standard deviations. If a normal distribution be assumed for the type of data presented herein, one may approximate to the 95% range by excluding the top and bottom 2½% of observed values. This is the "95% range" as used below. It should be stressed that the data are derived from 3 tests on each individual, and that both chemical and hematologic values are obtained from the same dogs.

Chemical Results. Urine. Distribution of urinary excretion of nitrogen is shown graphically in Fig. 1 and 2. Total nitrogen excretion is shown for 314 twenty-four-hour collection periods on 105 dogs. The shape of the distribution appears to be nearly normal; 60% of the samples contain between 6 and 9 g. Since the diet provides 7.6 g of nitrogen/day, most dogs would seem to be in nitrogen balance. Urea nitrogen excretion is less variable than total nitrogen. Two-thirds of the samples are in the range of 5 to 7 g of urea nitrogen/day. The difference between total and urea nitrogen excretion for individual samples was, in most cases, between 1 and 2 g/day. Excretion of creatinine was more variable than that of either urea or total nitrogen (Fig. 2). When the creatinine excretion for individual samples was plotted against urea excretion, a positive correlation was apparent. On the other hand, no correlation was observed between urine volume and amount of creatinine excreted, although such a relation is often assumed to exist in the human.

The excreted volume ranged from less than 200 ml to more than 1200 ml. The urine pH

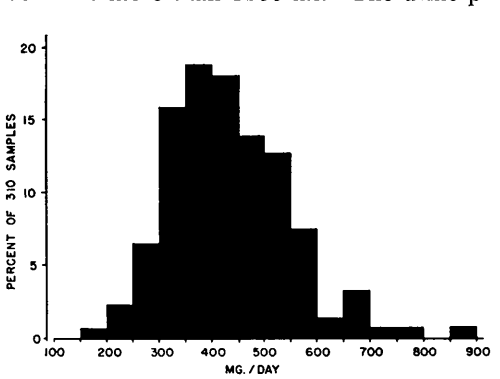

FIG. 2. Daily excretion of creatinine by normal dogs.

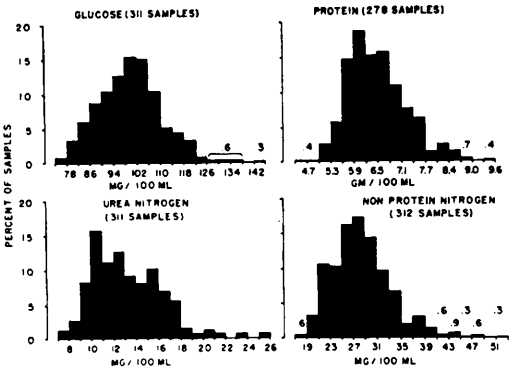

FIG. 3. Concentration in heparinized plasma of normal dogs.

was between 6.5 and 7.5 for 80% of the samples; the 95% range was 6.2 to 8.4. No positive bile tests were observed, and in only 5 samples was protein found by the acetic acid test. In 23 cases, positive Benedict's tests were seen. More than half of these were in one group of 12 dogs and are believed to represent an error of judgment in reporting as "positive" concentrations of less than 0.1 mg/100 ml.

Blood. Data for 4 common constituents of plasma are presented in Fig. 3. Sixty per cent of the glucose values were in the range of 90 to 110 mg/100 ml; 95% ranged from 80 to 120 mg/100 ml. The urea nitrogen distribution curve differed considerably from the statistically normal, but most values fell between 8 and 18 mg/100 ml. Two-thirds of the protein values fell between 5.6 and 6.8 g/100 ml. These limits are about one gram

TABLE I. Plasma Constituents Determined in a Limited Number of Normal Female Mongrels, Selected and Maintained as Described in Text. Except for cholesterol, 2 or 3 weekly samples were drawn on each animal.

Substance	No. of samples	Mean	"95% range"	
Bilirubin	69	.09	0 - 2	mg/100 ml
Cholesterol	20	193	136 -236	"
Creatinine	104	1.0	0.8- 1.2	"
Phosphorus, total	61	4.3	1.4- 6.5	"
Phosphatase, alkaline	61	4.2	1.7- 7.4	units
Potassium	24	4.1	3.9- 4.3	mEq/liter
Sodium	24	147	143 -150	"
Thymol turbidity	102	0.9	0 - 1.8	units

lower than have been reported elsewhere(1). The 95% range for non-protein nitrogen was 20 to 38 mg/100 ml. A plot of protein against non-protein nitrogen in individual samples showed no correlation between these components. The high values for glucose, urea, protein and non-protein nitrogen are scattered among the population and are not attributable to consistently high replicate values in a few animals.

A limited number of determinations of constituents listed in Table I has been made. The results may have lower validity than those of Fig. 1, 2 and 3, since a smaller number of dogs is represented and since all tests were not performed on a single group. Except for cholesterol, for which single values were obtained, the data of Table I include results of 2 or 3 weekly samples from each animal. In performing alkaline phosphatase determinations, it was found that the final pH of the buffered substrate-plasma mixture was actually between 9.6 and 9.8. Since it has been demonstrated(4) that the optimum pH is 9.5, these values are probably about 10% low.

Liver function was measured by the bromsulfalein test in 90 tests on 53 dogs. Retention at 30 minutes was less than 10% in all cases, and less than 3% in ¾ of the tests. Considerable data on kidney function in the dog have been presented in an earlier paper from this laboratory(5).

Hematologic tests. Fig. 4 summarizes results of hematology studies in 3 weekly control tests in over 100 dogs. The "95% range" is obtained by excluding the top and bottom 2½% of the values. Control data have been examined with respect to spread of the 3 weekly serial determinations on an individual dog. In general, the values for a particular dog did not vary greatly from week to week. Three hundred sixty *erythrocyte counts* were made on 120 dogs. The 95% range was 4 to 8 million/cu mm, but in only 11 dogs was the range of variation more than 1 million in the 3 weekly counts.

Hemoglobin varied more widely, the 95% range being 13 to 20 g/100 ml. In many dogs changes of 1 to 2 g were observed, but in a number of cases this was a consequence

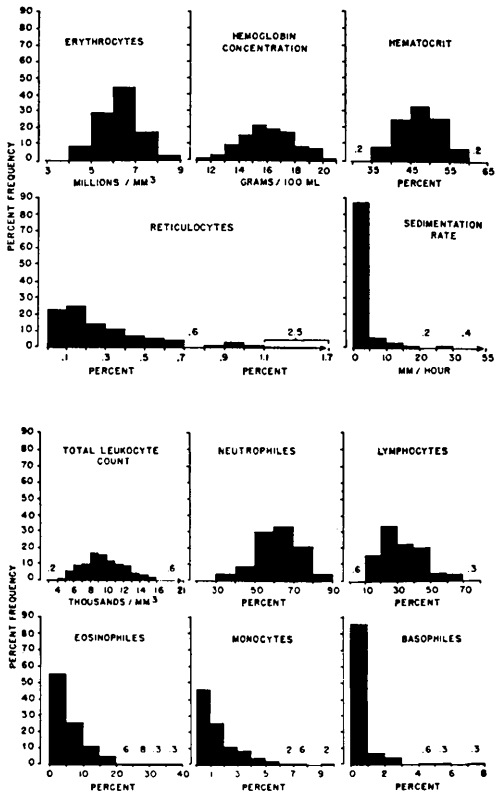

FIG. 4. Triplicate hematologic values in normal female mongrel dogs.

of increases in hemoglobin in dogs whose physical condition was obviously improving during the study period.

Hematocrit values are in accord with literature reports in this species. For any given dog, the 3 serial values were remarkably constant; in only 7 cases was the spread more than 10% among the 3 weekly determinations. The percentage of *reticulocytes* varied greatly, and a marked spread of values was often seen in individual dogs. The 95% range is meaningless in this instance; in one-half of the dogs, the count was less than 0.3% reticulocytes.

Sedimentation rates (Westergren) were zero in most cases. In 14 dogs, the sedimentation rate changed by 10 mm/hr from one week to another, but in one-half of these cases, the initial value was elevated and fell to normal in subsequent weeks.

The 95% range for *leukocytes* appeared to be from 5000 to 15,000/cu mm in this series. In general, the 3 determinations of total leukocyte count on an individual dog remained within 4000/cu mm, although in 3 dogs the count varied in a range of 8000/cu mm. A range of 9000 to 24,000/cu mm with a mean of 16,000 is reported in the literature(1). The lower values may reflect a lower incidence of intercurrent infection attributable to the selection and management of the dogs.

The percentage of *lymphocytes* and *neutrophils* ranged widely, with values as low as 50 neutrophils and as high as 50% lymphocytes in an appreciable portion of the population. For any particular dog, however, the percentage of either element changed only slightly from week to week; in only 10 cases was the range greater than 20%.

The *eosinophil* percentage was high in a few dogs, perhaps because of parasitism. Whether high or low, the count tended to remain constant at a value between zero and 10%. In only 8 dogs was the spread among triplicate values greater than 10%.

The range for *monocytes* was zero to 5%; within this range, a wider individual variation occurred from week to week than was anticipated. The 20 highest values were not restricted to a few dogs, although 4 dogs had more than 4% monocytes on 2 out of 3 counts.

A *basophil* count of zero was most frequent. The higher values represented sporadic high counts on a number of animals, rather than consistent elevation in a few.

Discussion. The present report defines the range of "normal" values to be expected within a large group of apparently healthy female dogs on a standard diet, using recognized procedures. The results are in general agreement with those compiled by Albritton(1) from a critical survey of the literature. A detailed comparison is beyond the scope of this report, involving questions of methodology, dietary states, and semantic difficulties associated with the word "normal."

An important feature of the ranges reported here for blood is their resemblance to those tabulated elsewhere(1,6) for the human. The "normal" distribution for most hematologic values is higher for the dog than for the human. Amounts of the chemically determined

constituents are similar to those of man. This would not necessarily be the case were other tests employed. Thus, in a number of samples in which thymol turbidity values were normal, the cephalin flocculation test was invariably positive, making the latter an invalid criterion for normal liver function in the dog. The differences may be a consequence of the dissimilar electrophoretic patterns that have been observed with canine and human serum.

The values observed for sodium and potassium in these "normal" dogs are higher than found in this laboratory for "trained" dogs that have been subjected to frequent venoclysis and venipuncture. It should be noted that whereas in the human the K^+/Na^+ ratio in erythrocytes is 220 times that in plasma, the K^+/Na^+ ratio in erythrocytes in dogs is similar to that in plasma. These considerations must be kept in mind in evaluating the importance of hemolysis in plasma samples and the significance of determinations of Na and K^+ in whole blood.

The differences in renal clearance values between dog and man and the closely associated differences in metabolism of drugs are illustrated abundantly in the literature and need not be summarized here.

Summary. Hematologic and clinical chemical data were obtained on 3 occasions in over 100 apparently healthy female mongrel dogs. Procedures are described and the normal ranges presented.

The authors wish to express their appreciation to members of the Pathology and Pharmacological Chemistry Departments, to whom should go much credit for the chemical and biological phases of this report.

1. Albritton, E. C., Ed., *Standard Values in Blood*, W. B. Saunders, Phila., 1952.

2. Hawk, P. B., Oser, B. L., and Summerson, W. H., *Practical Physiological Chemistry*, 13th Ed., Blakiston, N. Y., 1954.

3. Evelyn, K. A., *J. Biol. Chem.*, 1936, v115, 63.

4. Woodward, H. Q., *Cancer Research*, 1942, v2, 500.

5. Russo, H. F., Ciminera, J. L., Gass, S. R., and Beyer, K. H., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 736.

6. Sunderman, F. W., *Normal Values in Clinical Medicine*, W. B. Saunders, Phila., 1950.

Received February 6, 1957. P.S.E.B.M., 1957, v95.

Plasminogen in Pancreatic Disease and in Pancreatic Secretion. (23128)

ANNA B. BRIDGWATER AND H. NECHELES*
Department of Gastro-Intestinal Research, Medical Research Institute of Michael Reese Hospital, Chicago, Ill.

Plasmin is a proteolytic enzyme, normally existing in blood as inactive precursor, plasminogen(1). The latter may be transformed to plasmin by streptokinase(2) or other kinases(3-5). Plasminogen has been reported to be increased in a number of pathological and physiological conditions and, in some cases, blood has been found to possess spontaneous proteolytic activity, presumably due to presence of plasmin itself(1,6-14). In one report, excessive blood proteolytic activity was found in a patient undergoing surgery for carcinoma of the pancreas(15). Because several other serum enzymes are increased in pancreatic disease, it was thought of interest to investigate serum plasminogen levels when the pancreas was in an abnormal state. For the sake of clarity, several of the terms used are defined. *Plasmin.* Spontaneous proteolytic activity of blood; such activity is ascribed generally to plasmin. However, one should be mindful that characterization of this enzyme has not been sufficiently specific to rule out the possibility that this phenome-

* This investigation was supported by research grant from Natl. Inst. of Arthritis and Metabolic Diseases. The Department is in part supported by Michael Reese Research Foundation.