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Received November 22, 1955. P.S.E.B.M., 1956, v92.

Chemical and Hematological Studies on Blood of Bovine Dwarfs.*† (22531)

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Dwarfism in cattle has been reported since 1860 among many breeds of cattle in both *Bos taurus* and *Bos indicus*(1). Craft and Orr described an undersized Hereford steer with dwarf-like appearance and suggested this condition might be due to an underdeveloped thyroid and pituitary gland(2). A dwarf form morphologically similar to the preceding one has been studied more recently by various workers including Johnson, Harshfield and McCone(3), and Gregory and coworkers(4,5). This type of dwarf has a short broad head, an excessively bulging forehead, and marked prognathia. In addition to its small stature, it has a distended abdomen, a strong predisposition to bloat and breathes heavily. A premature fusion of the spheno-occipital synchondrosis in this dwarf type has been observed by Julian(6).

Due to the abnormal osteology in the dwarfs studied and their similarity phenotypically to mammalian cretins, this study of 38 "short-headed" dwarfs was initiated to understand more fully the pathological physiology present. Serum electrolytes and protein levels were run to aid in interpretation of osteological abnormalities. Cholesterol and protein-bound iodine determinations were used to assay thyroid function. Due to the lack of information on the hematology of the dwarf, blood studies were also included. The dwarfs were of both sexes and of both Hereford and Angus breeding.

Materials and methods. Samples were collected by jugular puncture prior to sacrificing for anatomical studies. All whole blood was collected in Heller and Paul's anticoagulant mixture for hematological studies. Standard methods were used for all blood counting procedures. Packed cell volumes were performed using Wintrobe's hematocrit tube. A Leitz Rouy Photometer was employed for hemoglobin determinations, using a wave length of 550 mμ, standardized by Wong's method for iron. Two hundred white blood cells were counted in all differential counts. Serum protein fractionations were carried out by the method of Wolfson, Cohn, Calvary and Ichiba(7) taking care to shake gently the span ether in the albumin determination. Saifer and Zymaris(8) have shown this prevents denaturation and approximates electrophoretic separation closely. Serum calcium was determined by the method of Clark and

* This study was supported by the Kellogg Fund of University of California for application of Genetics to Farm Animals.

† This study was conducted under Calif. Exp. Station Project 1451 in cooperation with the U.S.D.A. and State Experiment Sations under Western Regional Project W-1 on Beef Cattle Breeding Research.

‡ Authors are indebted to Dr. D. E. Jasper, Dr. L. M. Julian and Jane Bishop for assistance in gathering these data.

Collip(9), serum magnesium as by Levinson and MacFate(10), and inorganic phosphorus as recommended by Fiske and Subbarrow (11). A Coleman Junior Spectrophotometer model 6A was used for all spectrophotometric analyses. Cerebrospinal fluid was drawn by lumbar puncture and protein levels analyzed as by Johnson and Gibson(12).

Standard statistical methods were used and the mean ($\overline{X}$) and standard deviation (σ) are reported in all cases where values do not deviate from the normal. Deviations from normal in the blood differential counts also include standard error (ϵ), range (R), and random probability.

Results. The following data represent results from determinations on 38 Hereford and Angus dwarfs ranging in age from 6 days to 14 months of age: ($\overline{X} \pm \sigma$) cholesterol (156 ± 50.4 mg%), calcium (10.4 ± 1.2 mg %), phosphorus (7.6 ± 1.9 mg %), magnesium (3.25 ± 1.1 mg %), total protein (6.03 ± 0.9 g %), albumin (2.7 ± .67 g %), α globulin (0.74 ± 0.26 g %), β globulin (1.49 ± 0.55 g %) and γ globulin (1.05 ± 0.26 g %). Four serum protein-bound iodine determinations were 3.2, 3.7, 4.5 and 2.8 μg %.§ Five cerebrospinal fluid protein levels were 26.2, 22.8, 15.5, 28.0 and 55.0 mg%.

Hematological values are as follows: ($\overline{X} \pm \sigma$) packed cell volume (38.1 ± 0.5%), hemoglobin (11.7 ± 1.4 g %), W.B.C. (8.8 ±2.9 x 10^3/cmm), and R.B.C. (9.8 ± 1.6 x 10^6/cmm). In differential counts, lymphocytes are (%): $\overline{X}$ 55.6, σ ± 12.7, ϵ ± 1, and R 31-77. Neutrophils are (%): $\overline{X}$ 33.3, σ ± 12.5, ϵ ± 2.85 and R 10.5-58. Eosinophils are (%): $\overline{X}$ 2.1, σ ± 2.9 and monocytes (%): $\overline{X}$ 7.7, σ ± 3.5.

Discussion. Calcium, magnesium and phosphorus levels in the bovine dwarfs agree well with accepted normal values given by McSherry and Gryner(13) as well as those found in our own laboratory. Total protein and protein fractionations also appear to be normal. Both cholesterol and protein-bound iodine values are in the normal range and indicate that the "short-headed" bovine dwarf is not a primary thyroid cretin. Elevation in blood cholesterol levels could be expected if primary thyroid deficiency were present. Carroll *et al.* reported that a thyrotropic hormone deficiency was present in dwarf beef cattle(14). It is well known that hypopituitarism in rats is not associated with any marked change in plasma cholesterol levels (15,16). There still appears to be no obvious explanation for the fact that hypophysectomy does not cause an increased plasma cholesterol level as hypothyroidism in pituitary deficiency is very conspicuous. Protein-bound iodine determinations, therefore, appear to be better indices of thyroid function.

All hematological data agree with normal values of beef cattle under 14 months of age, as compiled by Schalm in our laboratory, except for the differential blood cell counts(17). Lymphocytes in normal beef cattle at this age are (%): $\overline{X}$ 68.0, σ ± 8.5, ϵ ± 1.6 and R 12-80. Neutrophils are (%): $\overline{X}$ 23.7, σ ± 7.7, ϵ ± 1.51, and R 12-38. The differences between normal and dwarf neutrophils and lymphocytes are highly significant with a random probability of less than 0.1% in both cases. Since total white counts do not vary from the normal, differences in the differential counts are not relative changes.

Summary. 1. Serum proteins, calcium, magnesium, and phosphorus of "short-headed" bovine dwarfs are all within normal limits. 2. Serum cholesterol and protein-bound iodine levels are within the normal range and indicate that the "short-headed" bovine dwarf is not a primary thyroid cretin. 3. All hematological values appear normal except for the differential count. Deviations from the normal are discussed.

§ Authors express their appreciation to Dr. A. Taurog, Dept. of Physiology, University of California, Berkeley, for protein-bound iodine determinations.

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Received March 1, 1956. P.S.E.B.M., 1956, v92.

Factors Affecting Extraction and Recovery of Follicle Stimulating Hormone From Sheep Pituitary Glands.* (22532)

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A method was reported previously for extracting gonadotrophin from dry sheep pituitary tissue with saturated solutions of NaCl and KCl at 70°C(1). Further work resulted in a short simple procedure for obtaining follicle stimulating hormone by extracting pituitary tissue with saturated NaCl solution, removal of a protein fraction from the extract at pH 2.5, dialysis to remove the salt from the soluble fraction, and recovery of the activity quantitatively by use of an anion exchange resin(2). This procedure provides a method for obtaining relatively large amounts of follicle stimulating hormone (FSH) essentially free of luteinizing hormone (LH). The basic steps in this procedure were studied to determine the optimum conditions for increasing the yield and/or biological purity of the FSH preparation. The factors studied were concentration of salt and pH during extraction, and temperature and pH during fractionation. These factors were varied as indicated in the procedure, and the results obtained are reported in this paper.

Materials. Acetone dried whole sheep pituitary tissue was the starting material for these experiments. The NaCl solutions used for extraction were 80, 90 and 100% saturated. The saturated solution was prepared by shaking for one hour a given volume of distilled water with an excess of NaCl. The 80 and 90% saturated solutions were prepared by diluting the saturated solution with the proper volume of distilled water. The anion exchange resin XE-59 and the cation exchange resin XE-97 were obtained from the Rohm and Haas Co., Philadelphia, Pa.

Method of preparation. The amounts of pituitary powder extracted varied from 10 to 100 g but in most cases either 50 or 100 g were used. The dry pituitary tissue was mixed with the salt solutions in the ratio of 1 g of powder to 10 ml of the proper concentration of NaCl solution. The mixture was adjusted either to pH 4, 5, 5.5 or 6 with concentrated HCl or to pH 7, 8, 9 or 10 with NaOH. The mixture was allowed to stand for

* Supported in part by research grant G-2154(C5) from the National Institutes of Health, Public Health Service, and by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.