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Distribution of Glucose in Blood of the Chicken. (22404)

DANIEL N. TAPPER AND MORLEY R. KARE.

Department of Physiology, N. Y. State Veterinary College, Cornell University, Ithaca, N. Y.

The whole blood sugar value for the domestic chicken varies over a wide range(1). A portion of this variation has been attributed to a sex difference. It has been reported(2) that immature fowls of both sexes and the mature hen have a similar glycemic level which is significantly higher than that of the adult male. Orchidectomy increases the whole blood sugar value to within the range of the hen(3). In a comparative study(4) with normal and testosterone propionate injected laying hens it was observed that androgen depressed the blood sugar level significantly. A sex variation in red cell volumes, counts, and hemoglobin values has also been reported for this species(5,6). Immature fowls of both sexes and the mature hen have a lower packed-cell volume, red cell count and hemoglobin percentage than the adult male. Coincident with maturation the cockerel shows a steady increase in packed-cell volume. The mature value represents an increase of approximately one-third. Sinistral poulards also have a high red blood cell value, whereas bilaterally ovariectomzied hens remain within the normal range for the hen(6). Androgen injection into immature chickens and into adult laying hens results in a marked and fairly rapid increase in red cell volume, red cell count, and hemoglobin level, all of which may attain the normal male adult range. Castration in the male results in values which do not significantly vary from those of the hen(7). This discussion suggests an intimate relationship between whole blood sugar levels and blood cell concentration. This association is supported by reports of an unequal plasma-cell glucose partition. One such report(8) for the hen indicates only 16.3% of the glucose in the circulating cells. Similar findings have been recorded in the pigeon(9). Little or no glucose has been found in the cells of swine, dog, cat, rat, guinea pig, and human(10,11).

It was decided to study plasma glucose levels in the chicken and to test the hypothesis that the sex variation reported for whole blood glucose values is a direct function of the cell concentration, and further that the androgen hypoglycemic effect is likewise a function of its red-cell-increasing capacity.

Materials and methods. Nineteen White Leghorn laying hens were used. Feed and water were offered *ad libitum*. Fifteen to 30 ml blood samples were taken in the morning after a 16-hour fast by heart puncture with a heparin-wetted syringe. The plasma was separated within 15 minutes. Packed-cell volumes were determined with Wintrobe tubes spun at 2500 rpm for 30 minutes. Since an error of approximately 10% is incurred by this low speed technic, high speed centrifuga-

TABLE I. Whole Blood and Plasma Glucose.

Group	Animals	Packed-cell vol	Whole blood, mg % glucose	Plasma, mg % glucose	% glucose in plasma	Calculated plasma, mg % glucose
Laying hens	12	30.7 ± .598*	172.3 ± 2.95	233.1 ± 4.38	93.8 ± .329	247.3 ± 4.51
Testosterone-inj. hens	7	40.3 ± 1.18	151.4 ± 2.22	225.0 ± 6.66	88.5 ± .640	254.6 ± 7.12

* Stand. error of mean.

tion was subsequently employed and correction factors were calculated. The force applied was above 6000 G. This procedure substantially reduces the inherent error in the method. Preparation of the filtrate and titration of the sugar was done by the Somogyi-Shaffer-Hartman method(12). Seven of the hens were injected intramuscularly every other day with 5 mg of testosterone propionate* in one ml of peanut oil for a total dose of 35 or 40 mg.

Results. The results in Table I show that the whole blood sugar level of the hens injected with testosterone propionate contains 20.9 mg% less glucose than the control laying hens. This difference is significant† at the one per cent level. There was no significant difference in the plasma glucose levels. The packed-cell volume of the injected hens is approximately one-third higher than that of the control hens.

Discussion. The whole blood sugar and packed cell values obtained agree with the reports describing a sex difference for the chicken. The mature hens showed a whole blood sugar value which was 12% higher than the androgen injected chickens. The injected hens showed a marked increase in circulating cells. But in both groups there was no significant difference between the plasma sugar levels. By subtracting the sugar referable to the plasma from the whole blood value, it may be seen that 88.5 to 93.8% of the circulating glucose is in the plasma and only a small fraction in the cells. Even this small amount may be due to *in vitro* changes in red cell permeability since it is known that once blood is shed glucose passes from the plasma into the cells and that anticoagulants increase the glucose permeability of the cells. Even with the use of heparin there is a slow passage of sugar into the cells. From limited trials it was observed that after 30 minutes approximately 85 per cent of the sugar remained in the plasma. In this respect it would appear that chicken blood resembles swine blood in which no transfer is observed after six hours as opposed to human blood in which passage begins immediately, is 50% complete within 10 minutes and completely equilibrated in 90 minutes(10,11).

The plasma-cell glucose partition observed in the chicken, coupled with the large difference in circulating blood cells found in the adults of the species, would explain the sex variation which has been described. As the cell concentration increases, the glucose in the whole blood is diluted. Whereas androgen has been reported to have a hypoglycemic effect, the above results show that androgen increases the number of circulating red cells but does not change the plasma glucose level.

In view of this plasma-cell partition, plasma glucose values are also shown in Table I as calculated plasma values. These were obtained by dividing the whole blood sugar value by the plasma volume percentage. This procedure circumvents the *in vitro* modifying factors by placing no restrictions on the use of anticoagulant nor by requiring plasma separation. Only whole blood and packed-cell volume determinations are needed.

Summary. Plasma rather than whole blood transports most or all of the glucose in the chicken. In view of variations in red cell concentration in this species, a measurement of plasma sugar can often be a more useful evaluation of circulating sugar concentration. The mature male and androgen injected females, which show a lower blood sugar level than immature fowls and laying hens, show

* Generously supplied by Schering Corp., Bloomfield, N. J.

† Students t applied.

no significant variation in sugar concentration of the plasma. It would therefore appear that the sex difference in blood glucose values reported for the chicken is a manifestation of variations in cell concentration. Androgen alters the whole blood sugar level by its cell-increasing action rather than by a primary action on sugar concentration.

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Low Molecular Weight Hexosamine-Containing Compounds in Urine.*† (22405)

NORMAN F. BOAS.

Department of Laboratories, Norwalk Hospital, Norwalk, Conn.

Hexosamines‡ are important components of connective tissue, synovial fluid, plasma and urine, and are frequently increased in amount in connective tissue diseases such as rheumatic fever, rheumatoid arthritis and disseminated lupus erythematosus(1-3). Hexosamines in the body are, almost exclusively, constituents of high molecular weight compounds (*e.g.* mucopolysaccharides, mucoproteins). They are not known to exist in a free state in nature(4) and even dialyzable hexosamine-containing compounds have not heretofore been demonstrated in body fluids and tissues.

It has been recently established by several investigators that a large non-dialyzable hexosamine fraction exists in normal urine(3,5,6). The development of an ion exchange method for the isolation of hexosamines(7) has made it possible to study quantitatively all hexosamine compounds in urine. During the course of such a study it became evident that a portion of the total urine hexosamine was freely dialyzable(3). Data are presented here which indicate that these substances form a relatively large pool of heretofore unknown low molecular weight hexosamine-containing compounds.

Methods. Urine specimens were collected for 24 hours and either studied directly or frozen until ready for analysis. Dialysis of urine was carried out at 4°C in large volumes of distilled water (1:50). The water was changed 2 times at 24 hour intervals. The difference between the concentration of hexosamine in the bag before and after dialysis (with appropriate volume corrections) was used to calculate the per cent of dialyzable hexosamine-containing compounds in urine. After acid hydrolysis and subsequent isolation

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‡ Naturally occurring hexosamines are glucosamine and galactosamine. The method used here does not distinguish between these two amino sugars.