ELECTROLYTES IN THE SERUM OF THE RAT*

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The use of the albino rat in biochemical studies other than those primarily concerned with nutrition is increasing. It is of importance, therefore, that data relating to the normal metabolism as well as to the chemical composition of body fluids and tissues be on record. There are in the literature only a few reports of the electrolyte content of blood serum of this species and these are more or less incomplete. In view of the fundamental significance of such information the following data are submitted.

EXPERIMENTAL

Animals and Diets—Male albino rats were obtained from the Connecticut Agricultural Experiment Station and from the Department of Physiological Chemistry, Yale University. The two colonies are from the same parent stock and are identically cared for and fed. Animals weighing at least 43 gm. at weaning (21 days of age) were given a diet of modified calf meal¹ (30), paste food,² and lettuce. The rats usually reached a weight of 115 gm. after 2 weeks on this adequate diet. Animals not weighing 115 gm. when 37 days old were discarded. When the desired body weight was attained (115 gm.), the rats were given a diet consisting of casein, 18 per cent; hydrogenated fat, 27 per cent; dextrin,

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¹ Modified by the addition of cod liver oil, 3 per cent.
² Whole milk powder, 25 per cent; casein, 25 per cent; wheat germ, 20 per cent; lard, 30 per cent.
51 per cent; and salt mixture (31), 4 per cent. This was supplemented by daily portions of yeast, cod liver oil, and wheat germ extracts as described by Swanson and Smith (43). The method of caging and the care of the animals followed the directions of Smith, Cowgill, and Croll (39). The growth of the rats was similar to that of the "normal control" animals described by Brooke and Smith (7).

Anesthesia and Collection of Blood Sample—The collection of arterial blood from rats without loss of carbon dioxide can best be accomplished when the animals are anesthetized. Although anesthesia is probably always accompanied by some variations in the composition of the blood, the available evidence indicates that urethane administered by stomach tube produces only minor disturbances in this respect (1, 3, 33).

In the present study urethane was given by stomach tube in doses of 2 gm. per kilo of body weight. Anesthesia was usually produced in 1 hour, during which period the body temperature fell about 1° unless otherwise prevented. To minimize possible changes in the water and electrolyte concentration due to this circumstance, the animals were kept in an incubator at 37° during this time. To determine the effect of the anesthesia analyses of chloride, carbon dioxide, and pH, and a few analyses of the base fractions were made on the blood serum of rats from which the blood was obtained by heart puncture.

1½ hours after the anesthetic was given, the peritoneal cavity was opened by an incision along the linea alba, and the abdominal aorta exposed. The aorta was held momentarily while entrance was made with a sharp hypodermic needle connected to a centrifuge tube by rubber and glass tubing, the whole of the tubing being filled with mineral oil. This is essentially the method described by Van Slyke and Cullen (46) for collecting blood without loss of carbon dioxide. 6 cc. of blood could be obtained in this manner from a single rat. The glass tubing was removed from the centrifuge tube, mineral oil was added to fill the tube, and a special rubber stopper containing a hypodermic needle was inserted until all the air was displaced. The needle was removed and the blood sample allowed to clot in the ice box. It may be that under these conditions there is a small loss of carbon dioxide (29).
Body Temperature—This was obtained by inserting a clinical thermometer 3 cm. into the rectum of the rat and leaving it in place until the temperature had reached a maximum. Feces were first expressed from the colon. The temperatures were corrected by calibrating the clinical thermometer with a standard laboratory thermometer reading to 0.01°.

Hydrogen Ion Concentration—The pH value of the serum was determined potentiometrically by means of a calomel half-cell together with a quinhydrone electrode, of the type used by Cullen and Biilmann (11) as modified by Cullen (10) and Cullen and Earle (12). By this method serum is admitted to the quinhydrone half-cell and electromotive force readings are made at definite intervals of time. These observations are plotted on coordinate paper with time as the abscissæ, and an extrapolation made to the time the serum is admitted; from this an apparent value for the pH is obtained. This value is subject to two corrections: the first obtains because the calculated apparent value differs from the true value as found with a hydrogen electrode at the same temperature; the second correction depends upon the difference between the temperature of the electrodes and the normal body temperature of the group of rats under investigation. These corrections for rat serum were obtained by comparing the apparent pH at different temperatures obtained with the quinhydrone electrode with the pH given by a glass electrode at 38° (13). The corrections for rat serum are similar to those given elsewhere (27) for dog serum.

Carbon Dioxide Content—The carbon dioxide in the serum was determined in the manometric gas apparatus described by Van Slyke and Neill (47). The determinations were made in duplicate on 0.2 cc. samples. The factors of Van Slyke and Sendroy (48) were used.

Chloride—Chlorides were determined in duplicate on 0.2 cc. samples of serum by Patterson’s modification (32) of the Van Slyke method (45).

Total Protein—Total nitrogen determinations in 0.5 cc. samples of serum were carried out in duplicate by a modified semimicro-Kjeldahl method similar to that described by Hitchcock and Belden (21) except for the use of selenium oxychloride as the catalyst,

* We are indebted to Mr. Delafield DuBois for these measurements.
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as used by Lauro (28). A few determinations of the non-protein nitrogen were made by direct Nesslerization, according to Folin and Denis (17), with persulfate as the oxidizing agent (51). The mean result of these determinations (0.25 gm. of nitrogen per liter) was used in calculating the values for protein nitrogen from total nitrogen. The usual factor, 6.25, was employed to estimate the total serum proteins. The ratio of albumin to globulin was not determined.

Inorganic Phosphate—The modified colorimetric method of Kuttner and Cohen (25) and Kuttner and Lichtenstein (26) was used to determine the inorganic phosphate in 0.2 cc. samples of serum. Bodansky (5) has shown that the blue color of the phosphomolybdate solution does not obey Beer's law and that a further error is introduced if trichloroacetic acid is used in the unknown and not in the standard solution. These errors can be estimated from tables which Bodansky has prepared. In the present study the error was reduced by using trichloroacetic acid in both the unknown and the standard solution and by preparing standard solutions of almost the same concentration as the unknown.

Calcium—The calcium in the ash of 1 cc. of serum was precipitated as the oxalate, filtered, and washed according to the method of Stanford and Wheatley (41) and measured by the gasometric method of Van Slyke and Sendroy (49). The filtrate was used for the determinations of the other base fractions.

Magnesium—The method of Strebinger and Reif (42) as applied to blood serum by Greenberg and Mackey (18) was used for the determination of magnesium on the filtrate from the calcium oxalate.

Potassium—The potassium in the filtrate from the magnesium hydroxyquinolinate was determined by the method of Shohl and Bennett (36) as modified by Hald (19).

Sodium—The sodium in the filtrate from the potassium determination was determined by the gravimetric method of Barber and Kolthoff (2) and Kolthoff (24) as applied by Butler and Tuthill (8).

Total Base by Benzidine Method. Total base was determined by the method of Stadie and Ross (40) as modified by Kirjan (23).

Total base was also determined by adding together the values for the individual cations.
Results

The small volume of blood obtainable from one rat does not admit of a complete electrolyte study on a single animal. In order that the data obtained from the numbers used might be interpreted

<table>
<thead>
<tr>
<th>Electrolytes in Serum of Normal, Adult, Male Rats</th>
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<tbody>
<tr>
<td><strong>Body temperature</strong></td>
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<tr>
<td><strong>pH of serum</strong></td>
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<tr>
<td><strong>CO₂, mm per l.</strong></td>
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<tr>
<td><strong>Chloride, m.-eq. per l.</strong></td>
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<tr>
<td><strong>Total protein, gm. per l.</strong></td>
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<tr>
<td><strong>Inorganic phosphate, mm per l.</strong></td>
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<td><strong>Ca, m.-eq. per l.</strong></td>
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<td><strong>Mg, m.-eq. per l.</strong></td>
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<td><strong>K, m.-eq. per l.</strong></td>
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<tr>
<td><strong>Na, m.-eq. per l.</strong></td>
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<td><strong>Total base by summation of cations, m.-eq. per l.</strong></td>
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<tr>
<td><strong>Total base by benzidine method, m.-eq. per l.</strong></td>
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* No anesthetic used. Blood obtained by heart puncture.
† Venous blood serum.
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properly, it was necessary to perform a sufficient number of determinations to make the mean value of a given blood constituent fairly reliable as evaluated by the statistical methods ordinarily employed in biological work (14, 16). The formulæ used were standard deviation = \( \sqrt{\sum d^2/(N-1)} \), where \( \sum d^2 \) is the sum of the squares of the deviations from the mean, and \( N \) is the total number of observations. Probable error of mean = \( (0.6745 \times \text{standard deviation})/\sqrt{N} \).

A summary of the results is contained in Table I. For purposes of comparison some values heretofore recorded in the literature are given, in some cases recalculated to the same denominations herein employed.

**DISCUSSION**

The blood serum obtained from rats by heart puncture without anesthesia does not differ appreciably in composition from that obtained under urethane anesthesia except as to chloride and carbon dioxide content. It seems probable that the accelerated breathing of the struggling animal during the heart puncture would account for the lower carbon dioxide content and the consequent increase in chloride. Silvette's value (38) of 114.9 milli-equivalents per liter for chloride is appreciably higher than the mean value reported in this paper. We are not able to account for this very considerable difference. A recalculation of the data of Heller and Paul (20) reveals a difference between the total base and chloride in the serum of arterial blood of 69.2 milli-equivalents per liter, which is much greater than the analogous value (45.8) in the present study. It seems unlikely that the sum of bicarbonate, protein, inorganic phosphate, sulfate, and organic acids in arterial serum is as much as 69.2 milli-equivalents per liter. The smaller difference between chloride and total base in the present study is due principally to the higher chloride and the lower potassium and sodium.

Compared with the values considered as normal for human serum (19) it appears that the blood serum of the rat has a higher calcium, magnesium, and phosphate content and is lower in sodium, bicarbonate, and protein.

The present studies have demonstrated that as small an animal as the rat may be employed in certain investigations relating to
water and electrolyte distribution, particularly those in which it is desirable to restrict standardized animals to a relatively expensive synthetic diet.

BIBLIOGRAPHY

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