DAILY AND SEASONAL VARIATIONS OF THE BIOCHEMICAL BLOOD INDICES IN THE LOWER APES

Yu. P. Butnev

Laboratory of Biochemistry (Head, Professor A. V. Trufanov) Institute of Experimental Pathology and Therapy (Director, Doctor of Medical Sciences B. A. Lapin) AMN SSSR, Sukhumi

Presented by Active Member AMN SSSR V. V. Parin
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As A. D. Slonim, O. P. Shcherbakova and others have shown [7, 8, 11 - 17], apes show marked daily and seasonal physiological variations. O. P. Shcherbakova [14, 16] showed that the degree of illumination plays a critical part in the daily periodicity of several processes. These changes include the so-called motor activity [13 -17], metabolic rate [8], body temperature [13 - 16], arterial pressure [6], the morphology of the blood [5], and the chemical constitution of the urine [13]. In apes body temperature varies over the 24 hour period by 2.5°, rising to a maximum between 2 and 4 p.m., and falling to a minimum between 2 and 4 a.m. In the morning, the metabolic rate may be 25% [8] or even 48% higher than in the evening. I. S. Kanfor [3] studied the daily variations in the blood sugar when the animals were fed at 9 a.m., 12 a.m., and 5 p.m., which were the usual mealtimes for the breeding station, and during fasting. When no food was given, hypoglycemia developed rapidly, reaching its maximum value by 8 p.m. At night there was a very small increase of the blood sugar over the evening value. Under normal feeding conditions, blood sugar was higher by day than by night. The seasonal effect showed in variations of "motor activity" [17], of the blood cells [4], and of thermal regulation [7].

As K. M. Bykov and others [2] showed, an important part in the daily variation of physiological functions is played by the mutual relationships between cortex and subcortex. The physiological changes associated with the time of day and of year are closely linked to specific changes of the intermediate metabolic stages. The determination of the metabolic mechanisms controlling physiological functions constitutes an important practical and theoretical problem in medicine. As E. M. Cherkovich [10] showed, disturbance of the 24-hourly rhythm of physiological function caused by random variations of the periods of light and darkness may induce neuroses and the development of pathological conditions such as hypertonia or coronary insufficiency. In this connection it is of interest to study the metabolic changes associated with the physiological rhythms.

To detect any daily or seasonal metabolic variations, in the present work we have followed changes in the chemical constitution of the blood, and have measured the concentrations of sugar, cholesterol, residual nitrogen, ATP, inorganic and acid-soluble phosphorus, and plasma proteins.

METHOD

Blood sugar was determined by the Hagedorn-Jensen method, cholesterol by Levchenko's method, residual nitrogen by Asel's method, protein by the Robinson-Hogden method, ATP by the amount of inorganic phorsphorus formed after hydrolysis for 7 minutes in 1 N hydrochloric acid (ATP was precipitated from the trichloroacetate filtrate by the addition of mercury acetate) The inorganic phosphorus was determined after precipitation with magnesia N. N. V. mixture by the color reaction with eukonogen.

In the diurnal experiments, we used sexually mature male and female hamadryl baboons. During the experiments the animals were fed at the normal times, i.e. at 9.00 a.m. and at 1.00 and 5.00 p.m. Blood was taken from the ulnar vein at 9:00 a.m. and at 1:00 and 5:00 p.m. directly before feeding, and at 1:00, 5:00, and 9:00 a.m. of the following days. In one set of experiments, in order first to extinguish the orienting reaction to the experimental set-up, blood was collected at 10:00 a.m. and 5, 10, 15, 30, 60 and 120 minutes after the start of the experiment, just as in our investigation on the animals used as controls for the blood sugar curves taken after a quantity of sugar had been given [1]. The experiment was counted as starting at 12:00 noon. Blood was then taken at 4:00, 8:00, and 12:00 p.m., and 4:00, 8:00 a.m. and 12 a.m. on the following days. In between times at which blood was collected, the animals were set free in the cage in which they were normally kept. Blood sugar determinations were made from the blood samples taken a short time before the beginning of each day's experiment.

To study the chemical composition of the blood during the course of the year, two Rhesus macaque monkeys, three hamadryl baboons, and two green marmoset tamarins were used. All of the animals were males. Blood tests were made monthly, and the animals were maintained in the usual way. As we have shown previously [1], through extinction of the orientating reflex to the experimental set-up, some of the blood indices changed as repeated tests were made on a single animal. On this account, before the experiments to determine the effect of the season on blood constitution, the animals were adapted to the procedure of removing blood in order to extinguish this orientating reflex.

RESULTS

The diurnal variation in the chemical constitution of the blood of a hamadryl baboon is shown in Fig. 1 and in the table. As can be seen from Fig. 1, the blood sugar reached its minimum value at 8-9:00 p.m. In the experiments in which the orientating reaction was first extinguished, the maximum blood sugar level was reached at 4:00 a.m., and without extinction at 9:00 a.m., i.e., at the start of the experiments. The greatest divergence between mean values of the blood sugar over a 24-hour period in the two experiments was statistically significant. In the experiment in which the orientating reaction was first extinguished, taking blood a short time before the start of the daily experiment caused a temporary drop in the blood sugar level, but it recovered 1.5 hours after the start of the experiment. This hyperglycemic reaction is typically associated with the orientating reaction to the experimental set-up, and it becomes extinguished.

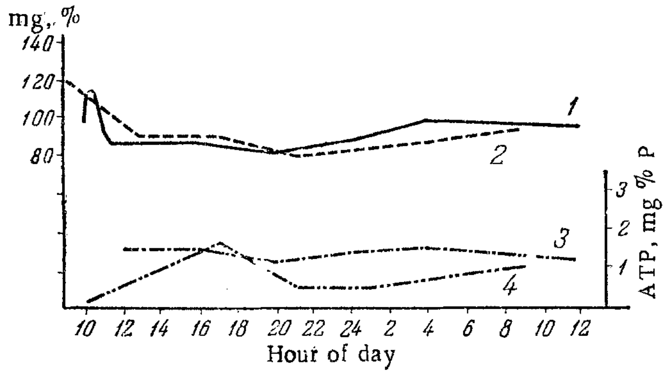

Fig. 1. Sugar and ATP content of blood of hamadryl baboons, showing variation over 24 hours. 1) Blood sugar in 24-hourly experiments, after previous extinction of the orientating reaction; 2) blood sugar in the 24-hourly experiments without previous extinction of the orientating reaction; 3) blood ATP, expressed as mg per cent of labile phosphorus; 24-hourly experiments, and previous extinction of the orientating reaction; 4) blood ATP, in 24-hourly experiments without previous extinction of the orientating reaction.

In both sets of experiments, the lowest blood sugar level occurred 4-5 hours after the last meal. However, despite the fact that no food was taken before morning, during the night the blood sugar gradually increased. Such changes can scarcely be attributed to the consumption of food. As L. S. Kanfor [3] showed in experiments on three apes, taking food at the normal times eliminates the most marked hypoglycemia, which developed during the second half of the daily experiment. However, in his experiments, in which the animals fasted, at night the blood sugar rose a few mg per cent over the evening level. We must note that in the diurnal experiments, the minimum blood sugar level occurred at the same time as the minimum metabolic rate, as determine by Q. P. Shcherbakova. Apparently, in apes the blood sugar is to a certain extent related to the rate of energy exchange. In this connection, the changes of such a high-energy compound as ATP are interesting. Although the variations of the blood ATP over a 24-hour period were not statistically significant, we must nevertheless note the reduction in mean level at the same time, 8:00-9:00 p.m. (Fig. 1). The results obtained in the two sets of experiments differ somewhat. Without extinction of the orientating reaction, there was some increase in blood ATP between the beginning of the experiment and 5:00 p.m. Apparently this increase was associated with the phenomenon of extinction of the orientating reaction. The minimum ATP in this set of experiments occurred at 1:00 a.m., and at 9:00 p.m. In the experiments in which the orientating reaction was first extinguished, the blood ATP was maintained at a more stable level. Some reduction occurred at 8:00 p.m. and at 12:00 noon on the following days.

The simultaneous reduction of the sugar and ATP at times when the metabolic rate was low shows that the concentration of these substances in the blood plays quite an important part in the regulation of energy exchange.

The results on the variation over a 24-hour period of blood cholesterol, plasma proteins, residual nitrogen, inorganic and acid-soluble phosphorus are shown in the table. The diurnal variations of these indices are statistical-

Content of Certain Components in Blood of Lower Apes over a 24-Hour Period

Blood components	Hour of day						
	12	16	20	24	4	8	12
Experiments with extinction of orientating reaction							
Cholesterol (mg %)	113±12.7	117±12.6	113±11.2	115±18.4	134±10.4	114±11.1	124±9.8
Residual nitrogen (mg %)	22±2.7	28±3.7	22±4,2	20±3.6	26±2.2	27±2.2	24±1.4
Plasma proteins (%)	7.1±0,43	6.7±0.45	6.7±0.42	7.1±0,49	7±0.39	7±0.47	7±0.26
Inorganic phosphorus (mg %)	1.19±0.23	1.46±0.35	1.08±0.23	1.12	0.84±0.11	0.96±0.18	0.97±0.23
Acid-soluble phosphorus (mg %)	13.2±2.8	15.11±1.7	17.08±1.2	14.6±2.4	16.09±1.9	15.2±1.4	13.41
	9	13	17	21	1	5	9
Experiments without extinction of orientating reaction							
Cholesterol, mg %	125±8.0	118±8.9	111±4.7	102±9.3	109±5.6	108±4.9	113±3.4
Residual nitrogen, mg %	18±1.3	17±1.7	15.2±1.8	18±2.5	19±0.6	18±1.5	18±0.9
Plasma proteins, mg %	6.9±0.16	7.2±0.24	6.9±0.11	7.3±0.16	7.1±0.13	7.4±0.18	7±0.16
Inorganic phosphorus, mg %	2.76±0.14	2.95±0.37	2.38±0.31	2.89±0.16	2.74±0.3	2.48±0.28	1.86±0.23
Acid-soluble phosphorus, mg %	16.27±1.3	21.31±1.1	21.16±0.7	19.81±1.4	19.4±0.9	18.93±1.0	19.77±1.2

ly insignificant. In the experiments in which there was no extinction of the orientating reaction, the cholesterol change followed that of the sugar. Minimum blood cholesterol occurred at 9:00 p.m., i.e. at the time when the metabolic rate and the ATP and blood sugar levels were minimal.

The results of the investigations into the seasonal changes of the chemical constitution of the blood (Fig. 2) show that blood sugar, cholesterol and ATP underwent regular changes. The variations of the blood sugar were statistically significant. In winter, the blood sugar fell, and ATP and cholestrol rose above the summer values. Residual nitrogen, inorganic and acid-soluble phosphorus and plasma proteins showed no regular changes related to the season.

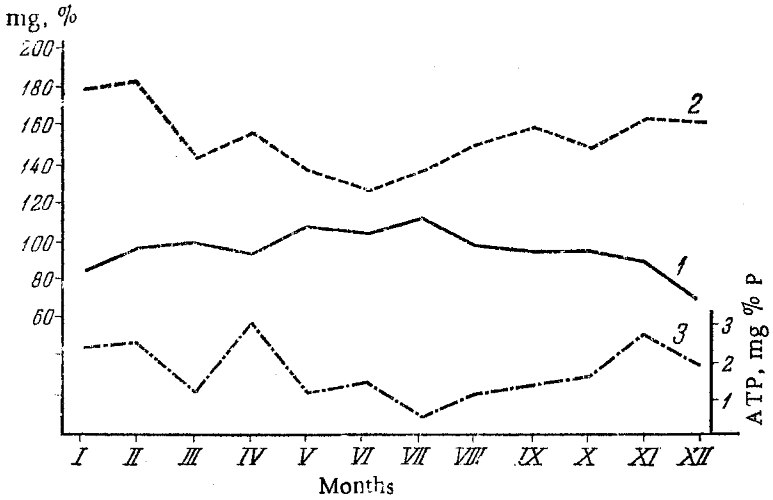

Fig. 2. Variation during one year in the lower apes of the blood content of (1) sugar; (2) cholesterol; and (3) ATP in mg per cent of labile phosphorus.

The increase of ATP in the blood during the cold season indicated that the resultant increase of energy production is brought about by an increase of tissue respiration associated with phosphorylation and not with any independent respiratory process. The increase of blood ATP in winter may explain the reduction of the blood sugar level, which might be due to increase of the hexokinase reaction, and to the consequently more rapid utilization of glucose by the tissues.

Thus the results of the studies of the changes in the chemical composition of the blood of apes in relation to the time of day and to the season confirm the suggestion that blood sugar and blood ATP play a definite part in the physiological mechanism for the regulation of the intensity of the energetic processes.

SUMMARY

Experiments were carried out on male and female hamadryl baboons daily throughout a 24-hour period. The blood sugar and blood ATP fell to a low level at 8-9:00 p.m. Concerning seasonal variation, we showed that the blood ATP and cholestrol blood contents increased during the cold season, whereas the sugar level dropped. The residual nitrogen, inorganic and acid-soluble phosphorus, and plasma proteins showed no regular seasonal or 24-hourly changes. Comparison of these results with variations of the metabolic rate in apes indicated that the blood sugar and blood ATP levels were possibly concerned in controlling energy changes in relation to external environmental conditions.

LITERATURE CITED

1. Yu. P. Butnev, Collection "Problems of the Physiology and Pathology of Apes". Sukhumi (1961).
2. K. M. Bykov, The Cerebral Cortex and the Internal Organs, Moscow–Leningrad (1947).
3. I. S. Kanfor, In book: An Experiment to Study the Periodical Changes of Physiological Functions in the Organism. [in Russian] Moscow (1949), p. 87.
4. M. I. Kuksova, In book: Theoretical and Practical Problems of Medicine and Biology in Experiments on Apes. Moscow (1956), p. 98.
5. M. I. Kuksova, Zhurn. obshchei biol., No. 1 (1959), p. 73.

6. G. O. Magakyan and D. I. Miminoshvili, Byull. éksper. biol. i med., No. 3 (1955), p. 27.
7. A. D. Slonim and R. A. Bezuevskaya, Fiziol. zhurn. SSSR, Vol. 28, No. 4 (1940), p. 330.
8. A. D. Slonim and K. P. Kalmykova, Transactions of the Sukhum Biological Station AMN SSSR, Moscow (1949), p. 18.
9. A. D. Slonim and A. R. Makarova, Transactions of the Sukhum Biological Station AMN SSSR, Moscow (1949), p. 22.
10. G. M. Cherkovich, Pat. fiziol. i éksper. ter. No. 6 (1959), p. 22.
11. O. P. Shcherbakova, Byull. éksper. biol. i med., Vol. 3, No. 4 (1937), p. 335.
12. O. P. Shcherbakova, Byull. eksper. biol. i med., Vol. 5, No. 2 (1938), p. 167.
13. O. P. Shcherbakova, Transactions of the Sukhum Biological Station AMN SSSR, Moscow (1949), p. 30.
14. O. P. Shcherbakova, Transactions of the Sukhum Biological Station AMN SSSR, Moscow (1949), p. 42.
15. O. P. Shcherbakova, In book: An Experiment to Study Periodical Changes of Physiological Functions in the Organism. [in Russian] Moscow (1949), p. 15.
16. O. P. Shcherbakova, In book: An Experiment to Study Periodical Changes of Physiological Functions in the Organism. [in Russian] Moscow (1949), p. 42.
17. O. P. Shcherbakova, In book: An Experiment to Study Periodical Changes of Physiological Functions in the Organism. [in Russian] Moscow (1949), p. 143.

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