remain in the egg-sack until next spring. 1st- and 2nd-instar spiderlings do not take any food, and no cannibalism occurs.

To discover a possible effect of photoperiod on the winter diapause of this spider, egg-sacks collected in the field in late September and kept in the outdoor conditions were periodically transferred to the laboratory conditions (20 °C, 70% rel. humidity) under various photoperiods (20 W white fluorescent lamp) as given in the table. 3 egg-sacks were kept under the outdoor conditions throughout the entire period as a control.

The results are summarized in the table. More than 95% of the spiderlings emerged in 26–39 days (average: 31 days) from egg-sacks which were kept in the long days over 13.5 h light:10.5 h dark since November 5, i.e., the day of the 1st moult. In the short days less than 13 h light:11 h dark, however, the percentage emergence was 5–31%, and 140–165 days were needed for the emergence. The critical day-length seemed to lie between 13 and 13.5 h in this season. Interestingly, it was shortened and occurred between 12 and 13 h when the egg-sack were transferred on December 15, 40 days after the 1st moult. From the egg-sacks transferred on February 4, over 90% emerged in 50 days even in 10 h light:14 h dark. Egg-sacks kept under the outdoor conditions throughout the entire period showed 99% emergence 160 days after the 1st moult.

The growth retardation at the 2nd instar thus seems to be diapause or a physiological state similar to that generally observed in overwintering insects. The main factor controlling this diapause seems to be a combined effect of temperature and photoperiod. A photoperiodic effect was clearly recognized before winter, but the critical day-length for the termination of diapause was changed during the overwintering period. The day-length in the early overwintering period (November) in central Japan, where the specimens were collected, is shorter than the critical day-length and shortened further until the winter solstice. Therefore, the diapause in autumn and early winter should be maintained by the short days. In mid winter, the emergence of the spiderlings seems to be inhibited by the coldness per se, while the suppressing effect of short days gradually decreases. This is effective to avoid the delaying effect of the spring short days.

The most interesting point in the present results is that the diapause in the grass spider is maintained by the short-day effect in autumn. Tauber and Tauber[9] cited similar examples from insects, among which the diapause character of the green lacewing, *Chrysopa harrisi* seems to be analogous to the present case. The critical day-length in the lacewing is gradually shortened during winter, and natural day-length in the spring is no longer significant as a releaser of diapause termination, being far longer than the critical value. The situation is similar in the spider, and the major function of its photoperiodic response would be to suppress the emergence of the spiderlings in autumn when the temperature is still above the lower limit for their active movement, and thus to ensure successful hibernation.

1 The author thanks to Dr K. Umeya for his encouragement, Prof. S. Masaki for his helpfull discussion, Dr Y. Tamaki and Dr K. Miyasita for providing necessary facilities for this experiment, Dr T. Yaginuma for his helpfull suggestion on the biology of the grass spider, and Prof. M. Schaefer for reading the manuscript.

2 A.D. Lees, in: The Physiology of Diapause in Arthropods. Cambridge, 1955.
3 C.D. Dondale and R. Legendre, C.r. Acad. Sci. Paris. *270*, 2483 (1970).
4 C.D. Dondale and R. Legendre, Bull. Br. Arach. *2*, 6 (1971).
5 M. Shaefer, Ent. Germ. *3*, 125 (1976).
6 M. Shaefer, Oecologia *25*, 155 (1976).
7 M. Shaefer, Z. ang. Ent. *83*, 113 (1977).
8 T. Yaginuma, in: Spider of Japan in Colour, p.206. Hoikusha, Osaka 1968
9 M.J. Tauber and C.A. Tauber, A. Rev. Ent. *21*, 81 (1976).

Features of intracellular calcium distribution in the adipose tissue of spontaneously hypertensive rats (SHR)

Yu. V. Postnov and S.N. Orlov

Central Research Laboratory of the Ministry of Public Health of the USSR, Timoshenko av. 23, Moscow 121359 (USSR), 19 January 1979

Summary. The in vitro study of the kinetics of ^{45}Ca efflux from adipose tissue of rats reveales 3 pools of exchangeable calcium. Calcium content in the intracellular pools of adipose tissue of spontaneously hypertensive rats is increased as compared to that in normotensive controls.

Abnormality in calcium handling by the cell membranes has been found in vascular smooth muscle[1–4], in cardiomyocytes[1,2] and in erythrocytes[5] of spontaneously hypertensive rats (SHR); this gives certain ground for considering these findings as fragments of a more wide-spread deficiency of the membrane maintenance of intracellular free calcium concentration. This membrane defect in SHR seems to be related, at least, to some types of tissues of mesenchymal origin; its possible presence in the cells of adipose tissue may explain the differences in the sensitivity of adipocytes to the in vitro action of insulin[6], ACTH[7] and adrenalin[8] recently found in SHR.

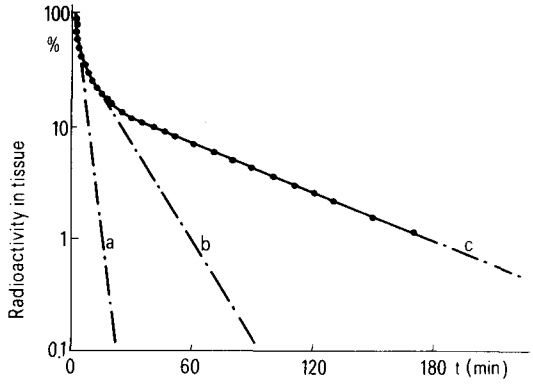
The present investigation was performed to study the features of intracellular calcium distribution in the adipocytes of SHR by the efflux of ^{45}Ca from the adipose tissue in vitro. Since the differences in hormonal sensitivity in a number of cases had been found earlier in a series of adrenalectomized rats[6–8], a part of the present experiments were carried out on previously adrenalectomized animals.

Material and methods. Spontaneously hypertensive 10-week-old rats (SHR, Kyoto Wistar) with blood pressures of 180–210 mm Hg were used. The normotensive rats (control group) consisted of inbred male Wistar rats (NWR) of the same weight and age (BP 80–100 mm Hg). All of the animals were kept under the same conditions on standard rat diet (briquettes) and tap water from the moment of weaning. A part of the study was carried out on rats adrenalectomized 7 days prior to the experiment. Following adrenalectomy the rats drank a 1% NaCl solution. The systolic blood pressure (BP) was determined without anaesthesia by means of tail plethysmography. Before decapitation the rats were starved 24 h with free access to water (or a 1% NaCl solution). After decapitation pieces (200 mg) of the epididymal fat pads were cut off, weighed and washed in saline solution, preincubated for 2.5 h at 37 °C in Krebs-Ringer phosphate buffer with the addition of $^{45}CaCl_2$ (0.2 μCi/ml), and washed 3 times with a saline solution for 1 min. In the following 170 min the pieces were passed through 29 scintillation vials each containing 1 ml of the buffer (t° = 25 °C).

Calcium distribution in the adipose tissue. S_A, S_B, S_C and τ_A, τ_B, τ_C is the size and time of exchange for pools A, B, C, respectively

Groups	Number of animals	Mean diameter of fat cells (μm)	Calcium pools (nmole Ca/g wet tissue)			Time of exchange (min)		
			S_A	S_B	S_C	τ_A	τ_B	τ_C
1. Control intact rats	12	37.4±0.7	138.1± 6.1	22.9± 0.6	15.5±1.1	3.49±0.21	20.9±1.9	249±16
2. Spontaneously hypertensive rats	14	38.1±0.8	136.9±14.1	52.7± 6.1	21.8±1.1	4.09±0.27	19.9±0.6	302±52
3. Adrenalectomized control rats	13	32.4±0.8	140.6± 7.9	33.3± 4.6	20.4±1.7	3.91±0.43	21.0±0.9	194± 8
4. Adrenalectomized spontaneously hypertensive rats	14	33.4±0.7	153.3±16.3	84.8±10.3	35.7±2.1	4.17±0.27	18.2±0.3	166±26
$P_{1,2}$				<0.0001	<0.001			
$P_{3,4}$				<0.0002	<0.00001			
$P_{1,3}$				<0.05	<0.05			<0.01

Mean values ± SE are given.



Kinetics of $^{45}Ca^{2+}$ efflux from the adipose tissue. a, b and c are calcium pools with essentially different values of exchange rate constant.

Radioactivity extruded from the tissue and radioactivity remaining in it after incubation for 170 min was determined on a scintillation-liquid counter (SL-4002, Intertechnique) with correction of the possible differences in the efficiency of the count. Plotting of kinetics of ^{45}Ca efflux in semi-logarithmic coordinates (figure) shows 3 exponents with essentially different time constants; this enabled us to use the equation suggested earlier[9]:

$$S_t = S_A \exp(-t/\tau_A) + S_B \exp(-t/\tau_B) + S_C \exp(-t/\tau_C),$$

where S_t is the amount of exchangeable calcium left in the tissue by the moment of time t, S_A, S_B, S_C and τ_A, τ_B, τ_C, is the volume and the time of exchange for the 3 calcium pools (A, B and C, respectively).

Results and discussion. The data on calcium content in the adipose tissue in the 3 pools (A, B and C), and the time of calcium exchange in these pools are given in the table. The time of calcium exchange in the A, B and C pools is in good agreement with the data of Kissebach et al.[10], which were obtained on isolated adipose cells. According to the data of this study, the most rapidly exchangeable A pool refers to calcium localized on the cell surface and to calcium of the extracellular space, while B and C pools refer to intracellular calcium[10]. The most slowly exchangeable C pool is evidently related to the calcium accumulated in the mitochondria of adipocytes, since the rate constant of its exchange ($k = 1/\tau_C = 0.0040$ min^{-1}) agrees well with the rate constant of Ca efflux from isolated hepatocyte mitochondria[11]. As it is seen from the table, the parameters of the rapidly exchangeable (extracellular) calcium pool (A) are the same for the adipose tissue of all groups of animals studied. The differences in calcium distribution in the adipose tissue in intact hypertensive and normotensive rats refer to intracellular B and Ca calcium pools (in the adipose tissue of SHR the value of these pools is respectively 2.3 and 1.4 times larger than that of the control). Adrenalectomy does not affect the extracellular calcium pool while the value of the B and C pools increases. The differences in the value of intracellular B and C pools between hypertensive and control rats are maintained after adrenalectomy, despite the essential change in their absolute value (in the adipose tissue of hypertensive adrenalectomized rats the value of the B and C pools is 2.6 and 1.7 times larger than that of adrenalectomized control rats). No significant differences were found in the time of calcium exchange in A, B and C pools between hypertensive and control rats, in the series of intact animals, as well as in the series of rats subjected to adrenalectomy. The differences in the values of B and C pools are not determined by the size of the cells: the diameter of adipose cells in intact rats is almost the same in the groups being compared; adrenalectomy, which causes diminution of the diameter of adipose cells by 12–14%, does not result in any difference between the groups.

We suggest that the revealed features of intracellular calcium distribution in adipose tissue of SHR may explain the enhanced sensitivity of their adipocytes to ACTH, which was demonstrated in experiments on isolated adipocytes by measuring the c-AMP content and rate of lipolysis (this rifference was found in both the intact and adrenalectomized rats)[7]. However, the enhanced adipocyte sensitivity to certain other hormonal agents (adrenalin[8], insulin[6]), which are less dependent on calcium, was revealed in SHR only in the series of preliminarily adrenalectomized animals. This is apparently due to the fact that, in the absence of the stabilizing effect of the corticosteroid hormones on the adipocyte membrane (after adrenalectomy), the difference in the size of intracellular pools between SHR and NWR increases even more (see table).

1 K. Aoki, N. Ikedo, K. Yamashita, I. Sato, K. Tazumi and K. Hotta, Jap. Circul. J. *38*, 1115 (1974).
2 C.J. Limas and J.N. Cohn, Circul. Res. *40*, Suppl. 1,62 (1977).
3 J. Wei Wu, R.A. Janis and E.E. Daniel, Blood Vessels *14*, 55 (1977).
4 T.T. Zsoter, C. Wolchinsky, N.F. Henein and L.C.Ho, Cardivasc. Res. *11*, 353 (1977).
5 Yu.V. Postnov, S.N. Orlov and N.I. Pokudin, Pflügers Arch. *379*, 191 (1979).
6 Yu.V. Postnov and M.B. Reznikova, Pflügers Arch. *379*, 187 (1979).
7 S.N. Orlov, N.I. Pokudin and Yu.V. Postnov, Cardiology (Moscow) *18*, 125 (1978) [in Russian].
8 Yu.V. Postnov and M.B. Reznikova, Bull. exp. Biol. Med. USSR *86*, 672 (1978) [in: Russian].
9 A.B. Borle, J. Membrane Biol. *10*, 45 (1972).
10 A.H. Kissebach, P. Clarke, N. Vydelinghum, H. Hope-Gill, B. Tulloch and T.R. Fraser, Eur. J. clin. Invest. *5*, 339 (1975).
11 L. Mela and B. Chance, Biochemistry *7*, 4059 (1968).