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Central Nervous System and Hibernation

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Introduction

Everyone who has attempted to make experiments on hibernating hibernators has encountered a major difficulty: when touched, the hibernator awakes with the greatest ease. It is the reason, for instance, why as simple a datum as the arterial pressure in hibernation has been measured for the first time in 1959 only, by LYMAN[1] on the ground squirrel. Till that time, it was not known at all.

If so, it is evident that hibernating hibernators are characterized by the persistency of a nervous excitability in spite of a deep hypothermia. In this respect, they differ absolutely from artificially cooled homoiotherms which are in deep anaesthesia at central temperatures up to 20°C higher than those which are normally found in hibernators of the Northern Hemisphere, in deep hibernation.

Moreover, when a normothermic, awake, hibernator is artificially cooled by immersion into cold water, one sees that it still breathes at very low temperatures (+5°C), while the artificially cooled homoiotherm shows a respiratory arrest when its central temperature falls down to about 18°C. This very ancient observation, made by WALTHER[2], has been confirmed by all those who have done similar experiments. The pupil of WALTHER, HORVATH[3], already ascribes the death of homoiotherms in hypothermia to asphyxia by failure of the nervous regulation of respiration; effectively, artificial respiration suffices to lower considerably the lethal temperature (estimated by cardiac arrest) of a cooled homoiotherm.

In the experiments published by one of us with RICHERT[4] the respiratory arrest of the rat in hypothermia occurred for a temperature of the basis of the brain of 15.6°C while hibernators, artificially cooled by immersion, in summer, still breathed with much lower central temperatures (Table I).

(At the end of the experiment, the animals were quickly dried. All these hibernators withstood this hypothermia and have lived for weeks or months after the experiment.)

The resistance of hibernators to hypothermia appears from Figure 1, on which are represented records of cerebral waves, electrocardiogram, cortical temperature and colonic temperature obtained in albino rats and some hibernators artificially cooled in a refrigerator, after curarization, under artificial respiration.

Evidently, one still records in hibernators a cortical electric activity at temperatures at which there is already an electric silence in homoiotherms (albino rat).

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[1] C. P. LYMAN, Fed. Proc. *18*, 96 (1959).
[2] A. WALTHER, Arch. Anat. Physiol. wiss. Med *1865*, 25.
[3] A. HORVATH, Pflügers Arch. ges. Physiol. *12*, 278 (1876).
[4] CH. KAYSER and R. RICHERT, C. R. Acad. Sci. (Paris) *246*, 2799 (1958).

Table I. Cardiac and respiratory frequency of normothermic and awake hibernators in summer rendered hypothermic by immersion

Date of experiment	Species	Weight (g)	Number of animals	Bath temperature (°C)	Colonic temperature (°C)	Respiratory frequency (beats/min) for the indicated temperature	Cardiac frequency (beats/min)
8.8.52	Garden dormouse (*Eliomys quercinus*)	70	2	5	6	60	72
29.8.52	Ground squirrel (*Citellus citellus*)	130	2	3	5	12	7
23.8.52	European hamster (*Cricetus cricetus*)	365	2	3.5	4.5	12	12

Awake and normothermic hibernators in summer are still distinguishable from homoiotherms by their remarkable resistance to anoxia. HIESTAND et al.[5] have shown that for an ambient temperature of 21°C, the sparrow resists only for 21 sec to a sudden barometric depression from 760 to 100 mm Hg (p O_2 = 21 mm Hg), the mouse for 28 sec, the pigeon for 48 sec, the rat for 1 min 14 sec, the guinea-pig for 2 min 54 sec. Hibernators such as golden hamster and the ground squirrel resist for 8 min 54 sec and 18 min 3 sec respectively, in the same conditions, while the bat survives for more than 1 h. But the case of the bat is special for it is already very hypothermic at an environmental temperature of 21°C. Now the ability to enter hypothermia is essential in the resistance to anoxia: the decrease of the oxygen consumption permits the prolongation of the survival time in anoxia. The survival time of the bat, a poikilothermic animal (HALL[6])[7] at a barometric pressure of 100 mm Hg is of 3 h at 19.5°C, 2 h 20 min at 21°C, 6 min 35 sec at 26°C and 5 min 54 sec at 30°C (HIESTAND et al.[5]). Inversely, the high respiratory exchanges of the sparrow and the mouse account for the very short survival time of these species.

Biochemical Researches on the Metabolism of the Cerebral Substance of Hibernators

(1) *The role of the glucid metabolism of nervous centres in the resistance to anoxia and hypothermia.* Is it possible to find a metabolic characteristic of the hibernator brain cell respiration? The researches we have undertaken on this subject have been inspired by old observations: in 1824, EDWARDS[10] had seen that the hibernators are not the only ones to resist to hypothermia and anoxia; the same is found in new-born homoiotherms such as kittens. These experiments were taken up again by REISS and HAUROWITZ[11], then by FAZEKAS, HIMWICH et al.[12], who found that new-born rats resist for 50 min to a dose of 50 mg KCN per 100 g fresh weight, while adult rats die already after 10 min exposure to the same dose.

The resistance of very young animals to anoxia is to be ascribed to a particular resistance of the brain, for KABAT[13] shows that in the dog, studied from the age of 8 to 142 days, the maximal duration of cerebral circulation suppression that leaves no functional disturbance of nervous centres progressively decreases during the development from 15 to 7 min.

SELLE and WITTEN[14], HIESTAND and NELSON[15] simplify the technique measuring the duration of gasping manifestations of the head separated from the trunk; they see that this duration reaches 30 min in the newborn rat, but does not go beyond 20 sec in the adult.

The researches of HIMWICH et al.[16–18] have given indications on the metabolic particularities of the brain substance of new-born animals which may explain this resistance: in 1932, HIMWICH and NAHUM[18] showed that the respiratory quotient (R.Q.) of the brain is 1.0. In 1939, HIESTAND et al. showed that the addition of glucose or lactate to the suspension liquid considerably increases the respiratory intensity of brain slices from very young animals. Lastly, in 1942, HIMWICH[17] saw that glucose increases the resistance to anoxia of the new-born animal, while the reduction of glucid reserves decreases it.

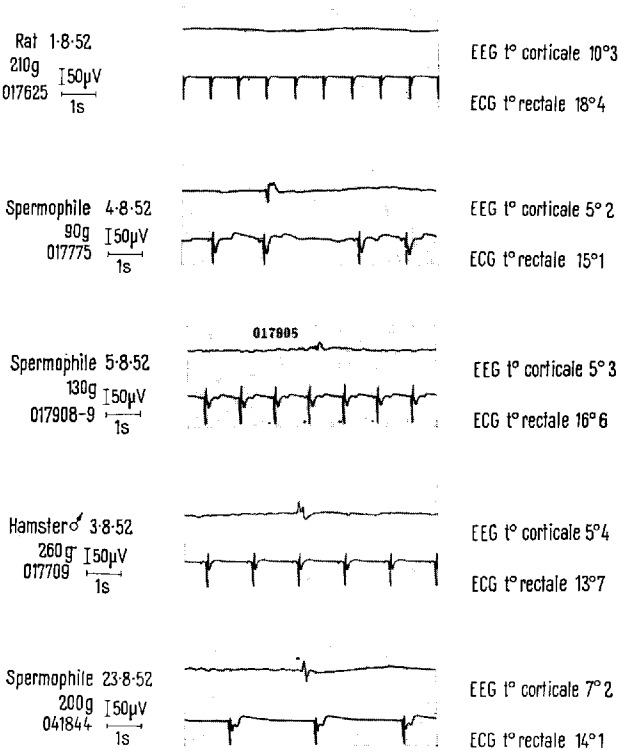

Fig. 1. Electroencephalographic and electrocardiographic records obtained during artificial hypothermia in a refrigerator, in albino rats and several hibernators.

[5] W. A. HIESTAND, W. F. ROCKHOLD, F. W. STEMLER, D. E. STULKEN, and J. E. WIEBERS, Physiol. Zool. *23*, 264 (1950).
[6] M. HALL, Philos. Trans. Royal Soc. London, *1832*, 335.
[7] For the discussion concerning the homoiothermia or the poikilothermal in bats in summer, see KAYSER[8] and POHL[9].
[8] CH. KAYSER, Ann. Physiol. *15*, 1087 (1939).
[9] H. POHL, Z. vgl. Physiol. *45*, 109 (1961).
[10] W. F. EDWARDS, *De l'influence des agens physiques sur la vie* (Crochard édit., Paris 1824).
[11] M. REISS and F. HAUROWITZ, Klin. Wschr. *1929*, 743.
[12] J. F. FAZEKAS, F. A. D. ALEXANDER, and H. E. HIMWICH, Amer. J. Physiol. *134*, 281 (1941).
[13] H. KABAT, Amer. J. Physiol. *130*, 588 (1940).
[14] W. A. SELLE and T. A. WITTEN, Amer. J. Physiol. *133*, P441 (1941).
[15] W. A. HIESTAND and J. W. NELSON, Amer. J. Physiol. *146*, 241 (1946).
[16] H. E. HIMWICH, Z. BAKER, and J. F. FAZEKAS, Amer. J. Physiol. *125*, 601 (1939).
[17] H. E. HIMWICH, A. O. BERNSTEIN, H. HERRLICH, A. CHESLER, and J. F. FAZEKAS, Amer. J. Physiol. *135*, 387 (1941/42).
[18] H. E. HIMWICH and L. H. NAHUM, Amer. J. Physiol. *101*, 446 (1932).

SELLE[19] and HIESTAND et al.[20] confirm HIMWICH. In the same year, CHESLER and HIMWICH measure the intensity of glycolysis and oxygen consumption *in vitro* of brain substance of rats aged 5 and 50 days; they find that at the age of 5 days, these two data have about one third of their value at the age of 50 days. To these low values they ascribe the resistance of cerebral substance to anoxia in the young animals; in fact, we have already seen that the sparrow and the mouse (HIESTAND[5]), which have very high respiratory exchanges, resist very badly to barometric depression.

Our own researches have been inspired by these experiments and by those of TYLER[21] who makes use of two poisons, malonate and monoiodacetate, and finds that: (1) Malonate lowers the oxygen consumption of new-born rat (aged 1 day) brain slices by 16.9%, while it lowers that of adult rat brain slices by 50% (2). Monoiodacetate lowers the oxygen consumption of new-born rat brain slices by 46%, that of adult rat brain slices by 26% only.

For TYLER, this action of monoiodacetate proves that new-born rat brain respiration depends more on the oxidation of products of glycolysis than does that of adult rat. He does not venture to draw any conclusion from his experiments with malonate: if he agrees to consider monoiodacetate as inhibitory for the 3-phospho-glyceraldehyde dehydrogenase, he does not venture to accept without reserve the specificity of action of malonic acid on succino-dehydrogenase.

In our experiments, one of us first tried to see if the influence of malonate on brain slices of new-born rats and of hibernators (European hamster, *Cricetus cricetus*) is the same. Studying the oxygen consumption of cerebral cortex slices of young rats from birth to adult state (1 year), one of us (KAYSER and LUCOT[22]) saw that in new-born rats it is about half (0.701 mm³ O_2/mg/h) of its value in adults (1.414 mm³ O_2/mg/h). Malonate has nearly no inhibitory effect on oxygen consumption in the new-born, while in adults it lowers it by 39%.

Comparing the effect of the same poison on the oxygen consumption of cortex slices of hamsters (*Cricetus cricetus*) and of adult rats, at 38°C, one finds no difference; in both cases, the reduction of the oxygen consumption amounts to about 36%—38% in the rat and 34% in the hamster. We find no difference similar to that observed between new-born and adult rats. But a highly significant difference appears when the same experiment is done at different temperatures, for instance 38°C, 23°C and 16°C (Table II).

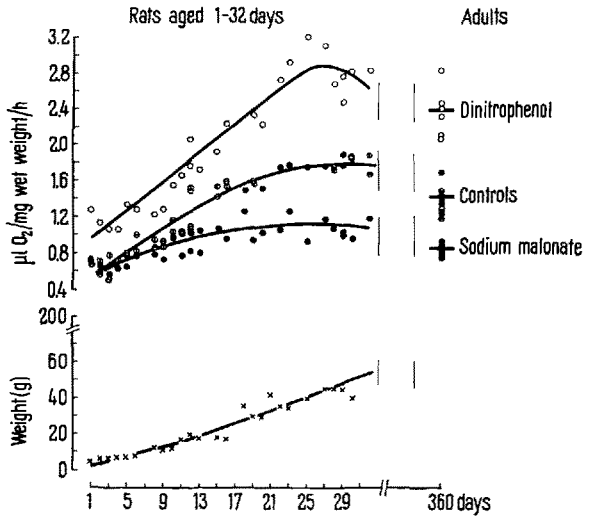

Fig. 2. Effect of malonate and dinitrophenol on the respiration of brain slices of rats during growth (Measurements done at 38°C; substratum: glucose)[22].

[19] W. A. SELLE, Amer. J. Physiol. *141*, 297 (1944).
[20] W. A. HIESTAND, R. D. TSCHIRGI, and H. R. MILLER, Amer. J. Physiol. *142*, 153 (1944).
[21] D. B. TYLER, Proc. Soc. exp. Biol. Med. *49*, 537 (1942).
[22] CH. KAYSER and M. A. LUCOT, C. R. Soc. Biol. *153*, 459 (1959).
[23] CH. KAYSER, Arch. Sci. Physiol. *15*, 377 (1961).

Table II. Combined effect of temperature and malonate (0.01 *M*) on the oxygen consumption of brain cortex slices of albino rats and European hamsters (*Cricetus cricetus*) (KAYSER[23], p. 404)

	38°C	23°C	16°C
Albino rat (mean body weight 206 g)			
Control slices	1.354 mm³ O_2/mg/h (N = 6; σ = 0.092)	0.408 mm³ O_2/mg/h (N = 6; σ = 0.045)	0.229 mm³ O_2/mg/h (N = 6; σ = 0.023)
Treated slices	0.855 mm³ O_2/mg/h (N = 6; σ = 0.032)	0.285 mm³ O_2/mg/h (N = 6; σ = 0.016)	0.150 mm³ O_2/mg/h (N = 6; σ = 0.027)
Reduction (%)	−36.9	−30.1	−34.5
Hamster (mean body weight 342 g)			
Control slices	1.251 mm³ O_2/mg/h (N = 6; σ = 0.053)	0.394 mm³ O_2/mg/h (N = 6; σ = 0.025)	0.178 mm³ O_2/mg/h (N = 6; σ = 0.037)
Treated slices	0.873 mm³ O_2/mg/h (N = 6; σ = 0.061)	0.344 mm³ O_2/mg/h (N = 6; σ = 0.062)	0.140 mm³ O_2/mg/h (N = 6; σ = 0.030)
Reduction (%)	−30.2	−12.7	−21.3
		F = 30.9	

The difference observed at 23°C is such that it brings about a significant specific difference between the two sets of 18 values, although the molecular activation heats computed for the two series show no significant difference (Table III).

The experiments with malonate show that it has a comparable effect in the hibernator and in the newborn rat: in both cases (studied with Alexander's test of trend) the reduction of oxygen consumption is less marked than in the adult rat.

Using the second poison used by TYLER, monoiodacetate, we do not find the same results: in our experiments, its effect is the same in the adult rat as in the hamster (Table IV).

It seems, therefore, that we have no right to ascribe to a single and identical cause the resistance to hypothermia and hypoxia of the nervous system of a newborn homoiotherm and that of an adult hibernator.

An evident difference between the respiration of brain slices of new-born homoiotherm and that of hibernator is the following: at 38°C, the oxygen consumption of hamster slices is only 6.5% lower than that of adult rat slices (1.251 or 1.272 mm³ O_2/mg/h against 1.354 or 1.344 mm³ O_2/mg/h) while that of newborn rat slices is 50% lower (0.701 mm³ O_2/mg/h against 1.414 mm³ O_2/mg/h) (KAYSER and LUCOT[22]).

These negative results led us to look for the glycolysis of rat and hamster cortex slices. The results obtained by comparing the oxygen consumption of rat and hamster brain slices at 38°C, on one hand, and the CO_2 production in a nitrogen atmosphere, on the other hand, did not allow us to observe any higher ratio of glycolysis/oxygen consumption in the hamster than in the rat (KAYSER[24]) (Table V).

We resumed these experiments, making an exact measurement of oxygen consumption, glucose consumption, and lactic acid production at 38°C and studying in parallel three tissues (brain, heart muscle, and kidney) of the rat and the hamster (PANTESCO et al.[25]). As Table VI shows, hibernator brain consumes more glucose and produces more lactic acid than homoiotherm brain, while such a difference cannot be found either for heart muscle or for kidney (Table VI).

[24] CH. KAYSER, *The Physiology of Natural Hibernation* (Pergamon Press, London, New York, Paris 1961).
[25] V. PANTESCO, M. A. LUCOT, P. MANDEL, and CH. KAYSER, C. R. Soc. Biol. *155*, 1709 (1961).

Table III. Molecular activation heat, between 16° and 38°C, for the respiration of cerebral cortex slices of albino rats and hamsters, treated with malonate

Species	Number of animals	Experimental conditions	Molecular activation heat (μ) in gcal	Slope (b)	Variance (V_b)	Comparisons
Rat	18	Control	14,564	3,166	11.101	Control rats/treated rats
Rat	18	Malonate	13,055	2,838	10.586	$t = 0.959$
Hamster	18	Control	15,745	3,423	19.856	Control hamsters/treated hamsters
Hamster	18	Malonate	14,540	3,161	51.659	$t = 0.985$

Comparison: Malonate hamsters/malonate rats: $t = 0.814$
Control hamsters/control rats: $t = 0.959$

Table IV. Combined effect of temperature and monoiodacetate (10^{-4} *M*) on the oxygen consumption of brain cortex slices of albino rats and European hamsters (*Cricetus cricetus*) (KAYSER[23], p. 403)

	38°C	23°C	16°C
Albino rat			
Control slices	1.344 mm³ O_2/mg/h (N = 5; σ = 0.154)	0.414 mm³ O_2/mg/h N = 5; σ = 0.032)	0.195 mm³ O_2/mg/h (N = 5; σ = 0.012)
Treated slices	1.084 mm³ O_2/mg/h (N = 5; σ = 0.047)	0.411 mm³ O_2/mg/h (N = 5; σ = 0.071)	0.183 mm³/mg/hg/h (N = 5; σ = 0.024)
Reduction (%)	−19.0	0.0	−6.1
Hamster			
Control slices	1.272 mm³ O_2/mg/h (N = 5; σ = 0.107)	0.443 mm³ O_2/mg/h (N = 5; σ = 0.023)	0.188 mm³ O_2/mg/h (N = 5; σ = 0.022)
Treated slices	1.067 mm³ O_2/mg/h (N = 5; σ = 0.103)	0.413 mm³ O_2/mg/h (N = 5; σ = 0.020)	0.168 mm³ O_2/mg/h (N = 5; σ = 0.018)
Reduction (%)	−16.0	−6.7	−10.6
		F < 1.0	

These results agree with those of HIMWICH, BERNSTEIN et al.[17], who pointed out the favouring effect of glucose on the resistance of new-born brain to anoxia.

(2) *Role of adenyltriphosphoric acid (ATP) in the functional particularities of the central nervous system of hibernators.* Considering the importance now ascribed to ATP in the energetic manifestations of cells, we have asked ourselves if some difference between hibernators and non-hibernators could be found in the oxidative resynthesis of this substance. Our first experiments made use of dinitrophenol, which is said to uncouple the oxidative phosphorylation of this essential constituent (LARDY and ELVEHJEM[26], HOTCHKISS[27]).

In a first experimental series, we have studied the action of dinitrophenol on the oxygen consumption of cortex slices of growing rats (Figure 2; Table VII).

In a second experimental series, we compared the effect of dinitrophenol on the oxygen consumption of cortex slices of albino rats and of hamsters. The findings (Table VIII) show that for a dinitrophenol concentration of $10^{-6}M$ the increase of oxygen consumption is a little more marked in the hamster than in the rat, but the difference is not statistically significant.

In our last experimental series, we have directly determined the intensity of ATP formation, simultaneously making an estimation of the efficiency of the reaction by measuring the P/O ratio. These researches were first done on brain substance homogenates and then on isolated mitochondriae. The results were the same in the two series (Table IX).

It results from these data that the intensity of ATP formation in the brain substance is greater in hibernators, in summer, than in homoiotherms. As the corresponding oxygen consumption does not differ from that of homoiotherms, the P/O ratio is higher in the

[26] H. A. LARDY and C. A. ELVEHJEM, Ann. Rev. Biochem. *14*, 1 (1945).
[27] R. D. HOTCHKISS, Adv. Enzymol. *4*, 153 (1946).

Table V. Effect of temperature on CO_2 production in a N_2 atmosphere, and on O_2 consumption in an O_2 atmosphere, of cortex slices of albino rats and European hamsters (*Cricetus cricetus*) (KAYSER[23], p. 401)

	38°C	23°C	16°C
Albino rat (mean body weight 392 g; experiment done in May)			
O_2 consumption	1.344 mm³ O_2/mg/h ($N = 5$; $\sigma = 0.154$)	0.414 mm³ O_2/mg/h ($N = 5$; $\sigma = 0.029$)	0.195 mm³ O_2/mg/h ($N = 5$; $\sigma = 0.012$)
CO_2 production	0.206 mm³ CO_2/mg/h ($N = 5$; $\sigma = 0.023$)	0.077 mm³ CO_2/mg/h ($N = 5$; $\sigma = 0.006$)	0.030 mm³ CO_2/mg/h ($N = 5$; $\sigma = 0.005$)
Ratio: glycolysis/O_2 consumption	0.126	0.186	0.154
Hamster (mean body weight 343 g; experiment done in April)			
O_2 consumption	1.272 mm³ O_2/mg/h ($N = 5$; $\sigma = 0.295$)	0.443 mm³ O_2/mg/h ($N = 5$; $\sigma = 0.023$)	0.188 mm³ O_2/mg/h ($N = 5$; $\sigma = 0.022$)
CO_2 production	0.227 mm³ CO_2/mg/h ($N = 5$; $\sigma = 0.013$)	0.134 mm³ CO_2/mg/h ($N = 5$; $\sigma = 0.013$)	0.037 mm³ CO_2/mg/h ($N = 5$; $\sigma = 0.008$)
Ratio: glycolysis/O_2 consumption	0.178	0.309	0.197
		$F < 1.0$	

Table VI. Oxygen consumption, lactic acid production and glucose consumption, at 38°C, of brain, heart muscle and kidney slices of albino rats and European hamsters (*Cricetus cricetus*) (PANTESCO, LUCOT, MANDEL, and KAYSER[25], p. 1711)

Species	Number of animals	Date of experiment	Tissue studied	Oxygen consumption mm³/mg/h	Glucose consumption γ/mg/h	Lactic acid production γ/mg/h
Rat	6	May 1961	Brain	1.506 ± 0.056[a]	4.576 ± 0.576	3.220 ± 0.122
Hamster	6	April 1961	Brain	1.442 ± 0.093	8.368 ± 1.007	4.291 ± 0.532
Rat	5	May 1961	Heart muscle	1.236 ± 0.043	4.189 ± 1.233	2.940 ± 0.303
Hamster	5	April 1961	Heart muscle	1.256 ± 0.047	3.835 ± 0.850	3.075 ± 0.443
Rat	6	June 1961	Kidney	2.781 ± 0.247	4.987 ± 1.818	1.453 ± 0.267
Hamster	6	June 1961	Kidney	2.891 ± 0.393	4.007 ± 0.859	1.382 ± 0.101

[a] Standard deviation

hamster; so ATP production is more intense, and the efficiency of the reaction better.

All these researches show that it is possible to find functional particularities, at the cellular level, of the brain substance of hibernators: glycolysis is more intense, glucose consumption, ATP production and P/O ratio are higher there than in homoiotherms.

Particularities of the Behaviour of Hibernating Hibernators due to the Persistency of Nervous System Excitability in Hypothermia

(1) *Thermoregulation in hibernation.* It is classical to say that the hibernating hibernator has lost its homoiothermia, that it has become a poikilotherm. In fact, its central temperature differs very slightly from the environmental temperature. And POPOVIC[28] has seen that the oxygen consumption of the hibernating ground squirrel (*Citellus citellus*) increases with the environmental temperature (these experiments have been made possible by the fact that the ground squirrel can remain hibernating even if the environmental temperature increases up to 20°C).

But we have known for 150 years (MANGILI[29], PRUNELLE[30], HALL[6]) that an intense cold awakes the hibernating hibernators. In our own researches, we have seen that there is an optimal temperature for hibernation, i.e. a temperature at which hibernation is the deepest and respiratory exchanges are the lowest.

[28] V. POPOVIC, Glas 208, Ac. Serbe Sciences No. 6, 43 (1952) (en serbe, résumé français).
[29] M. MANGILI, Ann. Mus. Hist. Nat. *10*, 434 (1807).
[30] C. F. PRUNELLE, Ann. Mus. Hist. Nat. *18*, 20, 303 (1811).

Table VII. Evolution during growth of the oxygen consumption of cortex slices of albino rats and its increase under the effect of dinitrophenol

Age of animals	Number of measurements	Mean body weight (g)	Oxygen consumption (mm^3/mg/h)		Increase of oxygen consumption under the effect of dinitrophenol (%)
			Control	In the presence of dinitrophenol	
1–5 days	5	6.3	0.735	1.139	55
6–10 days	4	10.9	0.941	1.342	43
11–15 days	5	18.5	1.253	1.833	46
16–25 dyas	6	31.4	1.666	2.620	57
1 month	5	49.6	1.787	2.792	56
3 months	3 (♂)	240.0	1.326	2.361	78
5 months	3 (♂)	345.0	1.259	2.237	78
7 months	3 (♂)	350.0	1.279	2.297	80
9 months	3 (♂)	365.0	1.245	2.133	71
12 months	6 (♀)	200.0	1.437	2.423	69

The measurements show that the effect of dinitrophenol is perhaps a little less marked during the first weeks of life (age 1–30 days) than later on (age 3–12 months). The effect is independent of the animal weight (12 months-old females: mean weight 200 g; 9 months-old males: mean weight 354 g) and of the intensity of the oxygen consumption (0.735 mm^3/mg/h on the first 4 days; 1.787 mm^3/mg/h between the 27th and 32nd day.

Table VIII. Combined effect of temperature and dinitrophenol ($10^{-6}M$) on the oxygen consumption of cortex slices of albino rats and European hamsters (*Cricetus cricetus*) (KAYSER[23], p. 405)

	38°C	23°C	16°C
Albino rat (mean body weight 207 g)			
Control slices	1.436 mm^3 O_2/mg[a]/h (N = 6; σ = 0.072)	0.411 mm^3 O_2/mg/h (N = 6; σ = 0.037)	0.229 mm^3 O_2/mg/h (N = 6; σ = 0.023)
Treated slices	2.424 mm^3 O_2/mg/h (N = 6; σ = 0.290)	0.813 mm^3 O_2/mg/h (N = 6; σ = 0.055)	0.420 mm^3 O_2/mg/h (N = 6; σ = 0.044)
Increase (%)	68.8	97.8	83.4
Hamster (mean body weight 360 g)			
Control slices	1.294 mm^3 O_2/mg/h (N = 6; σ = 0.111)	0.389 mm^3 O_2/mg/h (N = 6; σ = 0.035)	0.178 mm^3 O_2/mg/h (N = 6; σ = 0.037)
Treated slices	2.849 mm^3 O_2/mg/h (N = 6; σ = 0.213)	0.833 mm^3 O_2/mg/h (N = 6; σ = 0.031)	0.425 mm^3 O_2/mg/h (N = 6; σ = 0.051)
Increase	120	114	139

[a] Fresh weight.

$F = 1.46$ $(0.10 < p < 0.05)$

This temperature varies with the habitat: it is about 5–10°C for the European ground squirrel (*Citellus citellus*) and 15°C for two animals of Madagascar, the tenrec (*Centetes ecaudatus*) and a hedgehog (*Setifer setosus*). At temperatures lower or higher than this optimal temperature, the respiratory exchanges increase (Table X).

These experiments show that we may speak of a true 'regulation' at a minimum level of the energy expenditure in hibernation. This regulation, however, is but partial, and in the experiments of LYMAN[34] on the golden hamster (*Mesocricetus auratus*) all the animals did not react in the same way when the environmental temperature decreased during their hibernation.

Let us remark, also, that this reaction is also found in poikilotherms (KAYSER[35], TESTER[36]).

(2) *Diurnal and seasonal rhythms in hibernation.* HALL[6], JOHNSON[37], EISENTRAUT[38], UIBERALL[39], STRUMWASSER[40], have well seen that the beginning of hibernation manifests as a remarkable accentuation of the diurnal rhythm. In our researches on the artificial hibernation of the garden dormouse (*Eliomys quercinus*), one of us (LACHIVER and KAYSER[41]) has pointed out that the deep hypothermias occur in the morning and that in the evening the animals are awake and active.

Recent researches on this problem have been done especially by FOLK[42–44]. This author has stated that the arousals of the hibernating ground squirrel (lit for 8 h per day, from 9 a.m. to 5 p.m.) are more frequent by day than by night.

In our experiments on the oxygen consumption of the garden dormouse in deep hibernation we could observe no statistically significant difference between the oxygen consumption of the morning (38.9 ml/kg/h) and that of the evening (42.5 ml/kg/h) (KAYSER[24]).

[31] CH. KAYSER, G. VINCENDON, R. FRANK, and A. PORTE, Abstracts 2nd Internat. Symposium Natural Mammalian Hibernation, Helsinki, August (1962), in press.
[32] CH. KAYSER, C. R. Soc. Biol. *144*, 1697 (1950).
[33] CH. KAYSER, C. R. Soc. Biol. *154*, 1873 (1960).
[34] C. P. LYMAN, J. exp. Zool. *109*, 55 (1948).
[35] CH. KAYSER, Biol. Rev. *25*, 255 (1950).
[36] J. TESTER, Abstracts 2nd Internat. Symp. Natural Mammalian Hibernation, Helsinki August (1962), in press.
[37] G. E. JOHNSON, Quart. Rev. Biol. *6*, 439 (1931).
[38] M. EISENTRAUT, Z. Morphol. Ökol. Tiere *29*, 231 (1934).
[39] H. UIBERALL, Pflügers Arch. ges. Physiol. *234*, 78 (1934).
[40] F. STRUMWASSER, Amer. J. Physiol. *196*, 23 (1959).
[41] F. LACHIVER and CH. KAYSER, C. R. Soc. Biol. *152*, 1807 (1958).
[42] G. E. FOLK JR., Amer. Naturalist *91*, 153 (1957).
[43] G. E. FOLK JR., Bull. Mus. Comp. Zool., Harvard College *214*, 209 (1960).
[44] G. E. FOLK JR., Ann. New York Acad. Sci. *98*, 954 (1962).

Table IX. Oxygen consumption and ATP production of mitochondriae isolated from the brain of summer hibernators and homoiotherms (measurements done at 37°C; substratum: succinate; Warburg apparatus) (KAYSER, VINCENDON, FRANK, and PORTE[31])

Species and experimental conditions	Number of animals	ATP production (γmol/h/100 g proteins)	Oxygen consumption (γAtg/h/100 mg proteins)	P/O ratio
Hamsters (summer)	7	490 (59)[a]	232 (31)	2.11 (0.24)
Rats	7	395 (44)	208 (21)	1.61 (0.18)
Guinea-pigs	7	397 (73)	244 (42)	1.63 (0.17)
Rats + guinea-pigs	14	396 (66)	226 (37)	1.62 (0.17)
Results of statistical analysis				
Comparison summer hamsters/rats		$p < 0.01$	$p > 0.05$	$p < 0.01$
Comparison summer hamsters/guinea-pigs		$p < 0.05$	$p > 0.05$	$p < 0.05$
Comparison summer hamsters/rats + guinea-pigs		$p < 0.01$	$p > 0.05$	$p < 0.01$

[a] Numeral between brackets: standard deviation.

Table X. Oxygen consumption of three hibernators, hibernating at various temperatures (KAYSER[32,33])

Species	Body weight (g)	Environmental temperature (°C)	Oxygen consumption (ml/kg/h)	Number of measurements
Citellus citellus	185	10	19.6	10
	200	5	19.7	18
	160	2	38.9	10
Cententes ecaudatus	350	20	66.8	10
	350	15	39.1	13
	370	12	78.2	5
Setifer setosus	270	15	79.3	4
	253	20	205.5	4

We have resumed these researches on the ground squirrel (*Citellus citellus*), a diurnal animal, in opposition to the garden dormouse which is a nocturnal animal. We have studied 3 animals for 3 months; during the first month (from 15.12.62 to 15.1.63) they were lit from 6 a.m. to 6 p.m.; during the last two months (from 15.1.63 to 15.3.63) they were in total darkness. Thermistors were placed near the back of the animals; every arousal was expressed by a temperature increase and recorded. The records thus obtained show no diurnal rhythm for the beginning of the arousals; if we admit that night lasts from 6 p.m. to 6 a.m., we find 12 arousals by night and 14 by day (Table XI).

During the first month of observation (December 15 to January 15), during which the animals were lit for 12 h per day (6 a.m. to 6 p.m.), there were 8 arousals, 4 of which took place during the illumination period and 4 during the darkness period.

Our experiments—done, as a matter of fact, on a small number of animals—do not allow us to conclude a persistency of a diurnal rhythm of central nervous activity in deep hibernation.

POHL[9] studying the diurnal rhythm in bats, common dormice (*Glis glis*) and golden hamsters, has seen that if a diurnal rhythm may be found at the beginning of hibernation in certain species (bats), this rhythm progressively disappears as the hibernation becomes deeper.

The diurnal activity rhythm of poikilotherms is known to be independent of environmental temperature: the 'internal clock' is independent of the metabolic intensity which does undergo the influence of temperature and obeys the law of Van't Hoff-Arrhenius (FOLK[44], MARX and KAYSER[45]). Thus we could state that the period of activity of the lizard (*Lacerta muralis, Lacerta agilis*) is centred about 12.30 o'clock, be the measurements done at 19°C or at 29°C; on the other hand, the duration of the active phase varies from 3 h 30 min at 19°C to 10 h 50 min at 29°C, which corresponds to a Q_{10} of 3.0.

In observations made in the Far North, FOLK[44] found that the arctic ground squirrel (*Citellus parryi*) kept a diurnal temperature and activity rhythm in summer in spite of the constant illumination.

If we record the frequency of arousals of hibernating hibernators at two different temperatures, +6° and +10°C (KAYSER[35]), we find in 38 days 6 arousals at 6°C and 8 at 10°C; the frequency of arousals obeys the law of Van't Hoff, with a Q_{10} of 1.8.

If we find a diurnal rhythm only at the beginning of hibernation or of anticipated hibernation, provoked by a stay at a low temperature in June, the *seasonal* rhythm seems to have, at least in certain species, a high stability. In the ground squirrel, for instance, POPOVIC[31] could observe hibernation at 25°C, in winter. In spite of a high environmental temperature, the internal drive is sufficient to permit hibernation.

We have been able to follow tenrecs (*Centetes ecaudatus*) and hedgehogs (*Setifer setosus*) of the Southern Hemisphere for more than 3 years in our laboratory. Arriving from Madagascar in May, they began to refuse food in June. Seeing that, we lowered their environmental temperature from 20° to 15°C. The tenrec and the hedgehog then entered into hibernation and the measurements reported in Table X were done in July and August. In September they awoke, presenting at that time their best thermoregulation. They remained awake during the boreal winter; from September to May the temperature of their room was fixed at 20°C. During the boreal winter and spring their thermoregulation altered, becoming impaired. From May onwards they once more refused food and hibernated in a 15°C environment.

This seasonal cycle persisted unchanged for more than 3 years of observation in the same conditions, and still goes on.

(3) *Cerebral waves during hibernation.* If a hibernating hibernator is so easily awakened by light strokes, it means that its exteroceptive mechanoreceptors are able to give rise to sensory impulses which act upon centres able to answer by a thermoregulatory response.

All the hibernators do not seem to behave in the same way for all the exteroceptive receptors. Thus KAHANA, ROSENBLITH and GALAMBOS[46] could obtain no more action potential below 17°C on the auditory nerve in the golden hamster in hypothermia. One year later, CHATFIELD, LYMAN and PURPURA[47] studied the

[45] CH. MARX and CH. KAYSER, C.R. Soc. Biol. *143*, *1375* (1949).
[46] L. KAHANA, D. R. ROSENBLITH, and R. GALAMBOS, Amer. J. Physiol. *163*, 213 (1950).
[47] P. O. CHATFIELD, C. P. LYMAN, and D. P. PURPURA, EEG Clin. Neurophysiol. *3*, 225 (1951).

Table XI. Time of beginning of the periodic arousals of three ground squirrels (*Citellus citellus*) studied for three months (hibernation at 5°C from December 15, 1962 to March 3, 1963)

Hours	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12
No. of arousals	1	1	1	2	0	0	3	1	1	1	0	1
Hours	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24
No. of arousals	0	1	2	1	1	2	1	1	1	2	0	2

electrocorticogram of the hibernating hamster and observed a silence for cortical temperatures below 16°C; as a rule, spontaneous cortical electric activity manifested only for higher temperatures, between 19° and 21°C. They saw also that the cortex reacted to local strychninization only for temperatures higher than 16.4°C. These results, obtained during the hibernation of the golden hamster, agree with those obtained in the same species by artificial cooling under pentobarbital anaesthesia.

Studying the woodchuck (*Marmota monax*) LYMAN and CHATFIELD[48] recorded action potentials evoked by auditory stimulation when the cortex temperature had fallen to 7°C, and a spontaneous activity from 11°C onwards.

The much more recent results of STRUMWASSER[49] on a Californian ground squirrel (*Citellus beecheyi*) are almost opposite: he records a nearly uninterrupted cortical electric activity in hibernation (brain temperature 6°C) and considers it as a sign of the continuous thermoregulatory activity which keeps the brain at a constant temperature (±0.05°C) during hibernation.

Our own results differ from the first results of CHATFIELD et al.[47] and from those of STRUMWASSER[49], and are nearer those of LYMAN and CHATFIELD[48].

In our first researches (KAYSER[35], ROHMER, HIEBEL and KAYSER[50]) we had obtained movements of the legs of the ground squirrel (*Citellus citellus*) in deep hibernation by electric stimulation of the cortex. We had also observed the absence of any spontaneous cortical activity (brain temperature 5°C). Our observations, however, ranged only over short durations (2 to 20 min).

We resumed these experiments in 1961 (KAYSER[24]). It appeared then, on our graphs, that at 6°C (cortical temperature), one might record either a spontaneous cortical activity or silence. Spontaneous activity manifested itself, for a heart frequency of 3 beats/min, but only at the beginning of the experiments, i.e. 4 to 15 min after the installation of the animal. Later on, one recorded no spontaneous activity, but a noise (hand clap) elicited an electroencephalographic accident very comparable to that recorded by CHATFIELD et al.[47] in the same conditions and also showed great similarities with those which occurred spontaneously at the beginning of arousal in our records of 1951. 3 h after the beginning of the experiment, the same auditory stimulus elicited no more cortical reaction and we could record a cortical silence for up to 5 consecutive hours.

The graphs reproduced on Figure 3 originate from recent records. A ground squirrel (*Citellus citellus*) bearing chronic electrodes has been studied for about 1 month. The EEG recording was either continuous for 2 to 9 h, or automatically intermittent, for 2 min every 30 min, during several days.

In these experiments we have recorded arousals, entrances into hibernation and periods of uninterrupted hibernation. When the ground squirrel is hibernating at 14°C, electroencephalographic silence reaches only exceptionally 20 sec (Figure 3a). If it is hibernating between 5° and 10°C (Figure 3b), one may record either silence for hours or phases of slight electrical activity.

In one experiment only have we found records similar to those of STRUMWASSER: it concerned an animal by which the cement used to fix the electrodes formed a too important bulk which hindered skin cicatrization.

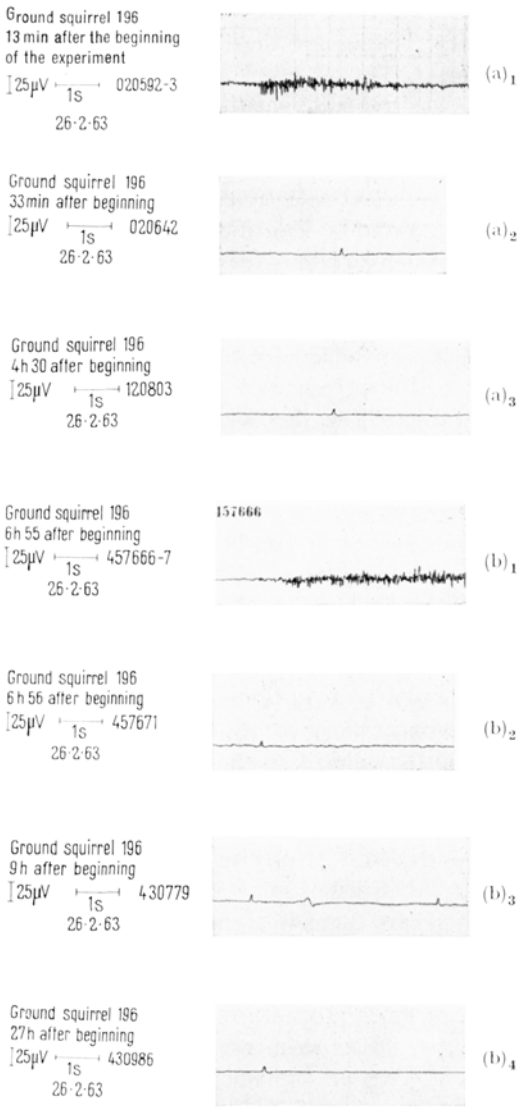

Fig. 3. Electroencephalographic records of a ground squirrel (*Citellus citellus*) hibernating at 14°C or at 7°C. (a) Hibernation at 14°C, (b) hibernation at 7°C (uninterrupted recording from 8.30 a.m. to 6 p.m.) (upward deflections on Figure 3 $(b)_{2,3,4}$ are hearts beats).

[48] C. P. LYMAN and P. O. CHATFIELD, Science *117*, 533 (1953); Physiol Rev. *35*, 403 (1955).
[49] F. STRUMWASSER, Amer. J. Physiol. *196*, 15 (1959).
[50] F. ROHMER, G. HIEBEL, and CH. KAYSER, C. R. Soc. Biol. *145*, 747 (1951).

In this animal, which presented only 3 days of continuous hibernation after the fixation of the electrodes, there was no electrocortical silence (Figure 4).

We conclude from our experiments that there may be an electroencephalographic silence for hours in a hibernator in deep hibernation. A light disturbance of the environment elicits an electrogenesis. There may also be an apparently spontaneous activity, which can be ascribed to no detectable external cause. Lastly, the same external stimulation may or may not elicit a cortical electric reaction. We have recorded neither a continuous total electrocortical silence, nor a continuous activity, lasting for the duration of a period of hibernation.

Cortical electric activity during arousal has been studied by us on several occasions[24,51]. LYMAN and CHATFIELD[52], CHATFIELD and LYMAN[53], RATHS[54] have analysed brain electric activity during arousal. Their researches allow to be identified the different parts of the brain which are progressively activated during arousal. According to the researches of CHATFIELD and LYMAN, the first electric activity (brain temperature 5.5°C) appears at the level of the basis pedunculi. They ascribe it to the presence of vegetative fibers in the pyramidal tracts. This precocious electrogenesis would express the strong emotive and affective manifestations of arousal. Very precocious also (7.5°C) is the activity of the cingulum. For these authors, the limbic system would be the first to be active, which seems to agree with the conceptions of MACLEAN on the role of the rhinencephalon (visceral brain).

The results of RATHS[54] are fairly near to those of CHATFIELD and LYMAN: he records the first activities (6°C) at the level of anterior mesencephalon, and later (9–10°C) at the level of rhinencephalon. He finds rather soon an activity at the level of lateral hypothalamus (10–12°C); on the other hand, the activity of mamillary bodies is very late (21°C).

There is, therefore, a precise time succession of the activation of the various systems in the central nervous system; it seems there is a sort of hierarchy in this activation.

Nervous regulations during the arousal manifest also in other fields: LYMAN and CHATFIELD[55], taking up again the old experiments of MARES[56] and DUBOIS[57], have given an explanation of the lag between the thermal ascension of the fore- and hind-parts of the hibernator during the arousal, showing that it is due to a vascular spasm, and insisted upon the fact that arousal is a strictly coordinated physiological process (1955).

The problem of entrance into hibernation is more complex: regulatory mechanisms occur together with manifestations of an 'overstraining' of the hibernator. The records made by LYMAN[58] along the 14 h of the entrance into hibernation of the woodchuck show that neither the temperature, nor the oxygen consumption, nor the heart frequency decrease regularly: at some times, the animal rewarms by increasing its oxygen consumption and accelerating its heart. It seems to want to resist hypothermia and sleep.

The records of STRUMWASSER[49] have given complementary information: they show 'plateaux' and 'steps' in the hypothermia which settles on the entrance into hibernation of the Californian ground squirrel (*Citellus beecheyi*) he studied. The 'plateaux', during which the temperature remains constant, show a slowing down of heat loss while the 'steps' reveal an increase of it. If LYMAN's records point out the 'resistance' to hypothermia, those of STRUMWASSER point out the active, regulatory part, expressed by the increase of heat loss, which alternates in the entrance into hypothermia and hibernation.

If the process of arousal thus appears as a perfect nervous integration, the entrance into hibernation seems to correspond to an imperfect regulation; it seems to be realized by 'rents' in a regulation.

In fact, we have seen more than 20 years ago (KAYSER[59]) that in hibernation certain central nervous regulations are not perfect: thus, when CO_2 concentration in the air is increased (up to 0.5 or 0.9%), a pulmonary hyperventilation does not always settle—as it does in normothermic state—so that hibernators

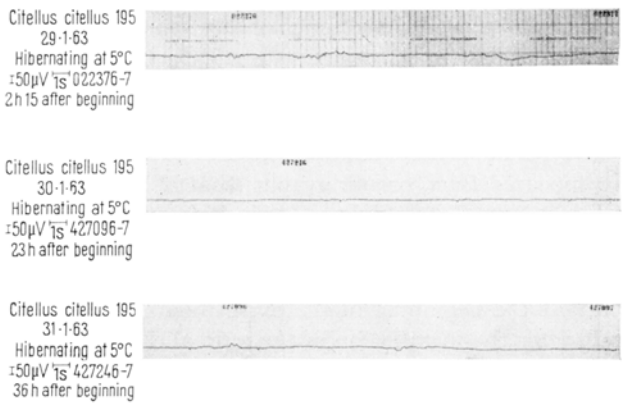

Fig. 4. Electroencephalographic record of a hibernating ground squirrel (*Citellus citellus*) by which the too large bulk of cement used for electrode fixation has prevented skin cicatrization. Temperature 5°C. (The small and rhythmic downward deflections are heart beats.)

[51] CH. KAYSER, Rev. Canad. Biol. *16*, 303 (1957).
[52] C. P. LYMAN and P. O. CHATFIELD, J. exp. Zool. *114*, *491* (1950).
[53] P. O. CHATFIELD and C. P. LYMAN, EEG Clin. Neurophysiol *6*, 403 (1954)
[54] P. RATHS, Z. Biol. *110*, 62 (1958).
[55] C. P. LYMAN and P. O. CHATFIELD, Amer. J. Physiol. *163*, 781 (1950).
[56] E. MAREŠ, C.R. Soc. Biol. *44*, 313 (1892).
[57] R. DUBOIS, *Physiologie comparée de la marmotte* (Masson édit., Paris 1896).
[58] C. P. LYMAN, Amer. J. Physiol. *194*, 83 (1958).
[59] CH. KAYSER, Ann. Physiol. *16*, 128 (1940).

may very easily show abnormal respiratory quotients in hibernation, when their respiratory exchanges are measured by the confinement method.

Conclusions. The role of the nervous system in the mechanism of hibernation is extremely important. The special properties of the brain of hibernators permits them wonderfully to resist to hypoxia and hypothermia, such as this 'state of *neural* hibernation', a reversible hypothermia which BULLARD, DAVID and NICHOLS[60] have obtained in the thirteen-lined ground squirrel (*Citellus tridecimlineatus*) by hypoxia at a low temperature.

The study of the glycolysis of the brain of hibernators has revealed that the brain of hibernators (European hamster) uses more glucose and produces more lactic acid than the brain of homoiotherms (albino rat). One finds, therefore, in hibernators two metabolic manifestations which are known to increase the resistance to hypoxia and hypothermia.

The study of ATP synthesis by isolated mitochondriae of the brain of hibernators shows that this synthesis is more intense in them than in homoiotherms.

The recording of cerebral waves indicates that during hibernation the cortex of hibernators remains excitable and manifests, either after external disturbances or without any apparent cause, periods of electrogenesis.

The role of the nervous system as an integrator of the arousal mechanism has clearly appeared in the researches on the electric activity of the various subcortical systems.

Hibernation appears as a metabolic regulation at a minimum level (WYSS[61]), a regulation in which the role of central nervous system is capital: its functional suppression, e.g. by anesthesia, leads to fatal hypothermias (BENEDICT and LEE[62], KAYSER[63], STRUMWASSER[40]).

Résumé. Le sommeil hivernal est une régulation métabolique à minimum (WYSS[61]). Cette régulation exige une température optimale de l'ambiance au-dessus et au-dessous de laquelle les échanges respiratoires de l'hibernant sont augmentés.

Le système nerveux central est responsable de cette régulation: si l'on supprime l'activité des centres nerveux de l'hibernant par des anesthésiques on supprime sa faculté de régler ses échanges et l'hibernant meurt en hypothermie (BENEDICT et LEE[62]).

Les centres nerveux de l'hibernant en sommeil hivernal restent excitables en dépit de la profonde hypothermie. L'hibernant partage cette faculté avec les homéothermes nouveau-nés, incomplètement développés. La résistance des centres nerveux à l'hypothermie se trouve associée dans les deux cas à une résistance accrue à l'hypoxie.

L'étude du métabolisme cellulaire de la substance nerveuse des hibernants et des mammifères nouveaunés révèle une consommation de glucose et une production d'acide lactique accrues. Vu que le blocage de la consommation de glucose et de la glycolyse supprime la résistance à l'hypoxie, il est naturel d'attribuer la résistance spéciale du système nerveux des hibernants et des jeunes mammifères à cette particularité métabolique.

L'intensité de la formation de l'acide adényl-triphosphorique par les mitochondries des cellules nerveuses des hibernants est aussi plus marquée que chez les homéothermes de même taille pris comme témoins. Cette seconde manifestation semble aussi correspondre à une adaptation particulière de l'activité des centres nerveux des hibernants à des conditions de fonctionnement spéciales.

Le sommeil hivernal ne s'explique pas uniquement par les particularités fonctionnelles du système nerveux des hibernants. Le sommeil hivernal est une manifestation saisonnière liée à des facteurs externes (température, lumière, nourriture) et à des facteurs internes (cycle saisonnier des glandes endocrines; KAYSER[59]). Mais seules les particularités fonctionnelles du système nerveux des hibernants autorisent une adaptation de l'hibernant aux conditions de vie en hypothermie. C'est la raison pour laquelle BULLARD et al.[60] ont pu réaliser chez un Spermophile américain ce qu'ils ont appelé le «Neural state of hibernation». C'est la même raison qui permet la survie de 30 jours en hypothermie du Lérot à jeun vivant à +5°C en été (KAYSER[23]), avec une dépense d'énergie qui ne diffère à peu près pas de celle du Lérot en hibernation en plein hiver.

[60] R. W. BULLARD, G. DAVID, and C. M. NICHOLS, Bull. Mus. Comp. Zool. Harvard College *124*, 321 (1960).
[61] O. A. M. WYSS, Pflügers Arch. ges. Physiol *229*, 599 (1932).
[62] F. G. BENEDICT and R. C. LEE, Carnegie Institution of Washington, Publ. No. 497 (1938).
[63] CH. KAYSER, Acta Neuroveg. *11*, 38 (1955).