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Short Communications

Seasonal Variations in Cytochrome *c* Content of Hedgehog Brown Adipose Tissue

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Many functions have been ascribed to brown adipose tissue,[1] which is very abundant in hibernators but is also found in non-hibernators such as the rat and the mouse.[1,2] Joel and Ball [3] found that brown adipose tissue from rats contains an abundance of various cytochromes, and Smith [4] reported that brown fat is a strongly thermogenic tissue. Further studies showing the important thermogenic effect of brown fat, especially in newborns, have been published by Dawkins and Hull.[5] This indicates that brown fat has an important metabolic function, which has recently been pointed out by Stratmann and Hohorst.[6] In the present paper the cytochrome *c* and water content of hibernating hedgehogs (body temperature 5–10°C) have been compared with that of non-hibernating animals (body temperature about 35°C). For a comparison some samples of brown fat from the rat, a non-hibernator, were analyzed for the same parameters.

Materials and methods. Samples were collected at different times during the year (Table 1) from hedgehogs (*Erinaceus europaeus*) kept under natural conditions at the General Hospital of Malmö. Non-hibernating adult hedgehogs were anaesthetized with 60 mg/kg b.w. of Nembutal injected intraperitoneally; the hibernating animals were operated on without any pretreatment. The brown adipose tissue was excised as completely as possible. The biopsies were sent to Stockholm by plane in thermos flasks (0°C) immediately after excision, stored at 0°C and analyzed individually the following day. Brown fat from white rats was collected from the interscapular region. Because of the small amount of brown fat obtained from the rats, material from 5–10 animals was pooled and analyzed. Cytochrome *c* was isolated from about 3 g of brown adipose tissue and its content was determined according to Paléus.[7] The $E^{1\,\%}_{1\,cm}$ 550 mμ of hedgehog cytochrome *c* was found to be 22.7, using a preparation obtained from three deep-frozen hearts. The wet weight, the dry weight and the lipid-free dry weight were determined according to Joel and Ball.[3]

Results. The total amount of hedgehog brown fat was higher during the hibernating period (December-March) and in May than during the non-hibernating period (Table 1). The water content and the lipid-free dry weight of hedgehog brown fat varied little, but slightly higher values did occur during the prehibernating (September) and hibernating (December-March) periods. The amount of cytochrome *c* was higher during hibernation and in May, but the concentration remained essentially the same. These results agree with those from another series of 14 hedgehogs from the hibernating and 16 from the non-hibernating period. In this series, the total weight of brown adipose tissue was higher ($P < 0.001$) during hibernation as were the water content ($P < 0.001$) and the total amount of cytochrome *c* ($P < 0.001$). In this larger series there was, however, a marked difference in the amount of cytochrome *c* expressed as mg/g lipid-free dry weight. Higher values were obtained during hibernation ($P < 0.05$), using the "t-test" to evaluate the results. The water content and lipid-free dry weight were lower in the brown fat from winter rats compared with the values from hedgehogs, but the cyto-

Table 1. Seasonal variations of water content and cytochrome *c* concentration of hedgehog and rat brown adipose tissue.

Date of collection of animals	Total wet weight in g	H_2O content in % of wet weight	Lipid-free dry weight in % of wet weight	Cytochrome *c* (mg) in total excised tissue	Cytochrome *c* in mg/g lipid-free dry weight
Hedgehog					
Jan. 20					
1	12.131	52	17.9	2.4	1.1
2	5.601	52	20.7	1.4	1.2
3	6.056	44	15.3	1.6	1.7
Mean	7.929	49	18.0	1.8	1.3
March 1					
1	12.057	55	19.0	2.3	1.0
2	10.238	53	17.7	1.6	0.9
3	8.735	47	16.7	1.6	1.1
Mean	10.343	52	17.8	1.8	1.0
May 10					
1	15.242	41	14.6	3.3	1.5
2	9.619	35	12.0	1.7	1.5
3	9.744	42	14.6	1.3	0.9
Mean	11.535	39	13.7	2.1	1.3
July 5					
1	3.427	49	14.8	0.6	1.1
2	6.975	44	15.2	1.4	1.3
3	4.617	39	12.4	0.7	1.2
Mean	5.006	44	14.1	0.9	1.2
Sept. 23					
1	4.673	50	17.5	1.1	1.4
2	4.378	42	13.6	0.7	1.1
3	3.626	48	13.1	0.7	1.4
4	4.515	49	14.7	1.1	1.6
Mean	4.298	47	14.7	0.9	1.4
Dec. 2					
1	11.817	52	19.7	4.4	1.9
2	9.830	57	17.7	3.3	1.9
3	11.541	50	15.9	2.0	1.1
Mean	11.063	53	17.8	3.2	1.6
Rat					
Jan. 19	2.894	26	7.0	0.2	1.2
Oct. 14	2.321	24	5.6	0.1	1.1
Dec. 16	2.605	20	6.0	0.3	2.2
Mean	2.607	23	6.2	0.2	1.5

The biopsies of September, October and December date from 1964, those of January, March, May and July 5 date from 1965.

chrome *c* concentration was about the same.

Discussion. Chaffee *et al.*[8] using brown fat of hamsters, found that the mass as well as the total mitochondrial content increased during cold acclimatization. Schollmeyer and Klingenberg [9] have shown that the cytochrome *c* content of mitochondria from different organs and species differs very little. Stratmann and Hohorst,[6] however, found that cold adaptation of newborn guinea-pigs did not markedly influence the cytochrome concentration of the mitochondria of the brown adipose tissue but rearing the animals 20 days at + 20°C caused a decrease of the cytochromes. Studies are now in progress to investigate the "mitochondrial density" of the brown adipose tissue of hedgehogs during a period of one year. The above results do not give information on the dependence of the variations on the light-dark and temperature cycles or other environmental factors.

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"Asterinsäure" — an Acetylenic Carotenoid

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"Asterinsäure", first isolated by von Euler and Hellström [1] from the starfish *Asterias rubens*, exhibits properties reminescent of astacene (*1*) [2] or astaxanthin (*2*).[3] Karrer and Rübel [4,5] later isolated a compound considered to be astacene (*1*) from the same source. The conversion of astaxanthin (*2*) to astacene (*1*) by alkali in the presence of oxygen has been demonstrated by Kuhn and Sørensen.[3]

Twenty years ago "asterinsäure" was isolated from the back skin of *Asterias rubens* by two of us (B.B. and A.H.) following in principle the procedure of von Euler and Hellström [1] involving precipitation of the chromoprotein with ammonium sulphate, cleavage of the chromoprotein with alcohol, partition, and finally crystallization from pyridine-water.

The compound isolated has been reexamined in recent years, and preliminary results were considered to indicate identity with astaxanthin (*2*).[6a] However, the evidence presented here shows that these compounds are different and that "asterinsäure" (*3*) is an acetylenic analogue of astaxanthin (*2*), either 7,8-didehydro-astaxanthin (*3a*) or a mixture of *3a* and the diacetylenic derivative 7,8,7′,8′-tetradehydro-astaxanthin (*3b*).

Bluish needles of *3* were obtained from pyridine-water (*2* crystallizes as plates from the same solvent pair [6b]); yield 0.88 mg, m.p. 215.5–216°C in evacuated tube (reported for "asterinsäure" m.p. 185°C [1] and for 2 m.p. 215.5–216°C [3]).

When special precautions were taken to avoid crystallization on the paper *trans 2* and *3*

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