Acetate and glucose utilisation and lipid synthesis by cold-acclimated and hypothermic rat-brain homogenates

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THE PHYSIOLOGY of cold exposure has been a favourite topic in the fields of comparative physiology and biochemistry (1). The effects of cold on the central nervous system has been under study for the past few decades. Growing interest in the application of acute hypothermia in surgery (2) has resulted in the study of thermogenesis of organ systems. Attempts at integration of the physiological response pattern to cold acclimation with tissues at cellular levels have found great difficulties. Though hypothermia has become a useful and extremely vital adjunct to cardio-vascular surgery and therapy of various diseased conditions (3), much of the biochemical changes occurring to the brain during hypothermia are still unclear.

In the **in vitro** studies reported in this paper, an attempt was made to reveal the incorporation of labelled glucose and acetate into lipids by cold acclimated and hypothermic rat-brain homogenates. When the rats were exposed continuously to cold for a period of two weeks, the term 'cold-acclimated rats' is used to denote such animals.

Materials and methods

Adult male albino rats, 14-16 weeks in age, reared in our own animal house and kept on normal rat pellets, were used for experimental studies. For cold acclimation, the rats were placed in straw-based cages and exposed to cold at 0-2° C. for 14 days. For control experiments, rats of the same age group and sex were kept at room temperature (29° C.). Both the control and experimental groups were fed **ad libitum** with normal rat pellets.

The reagents and solvents used in the experiments were all of analytical grade. Sodium acetate [1-^{14}C] and D-glucose [U-^{14}C] were both purchased from the Radiochemical Centre, Amersham, England.

Following the desired period of exposure in the cold room, the rats were immediately sacrificed by decapitation, the whole brain was carefully removed, completely blotted of blood and kept in chilled aluminium foils at 0° C. Using chilled vortex homogenization flask and ice-cold Krebs-Ringer phosphate buffer, pH 7.4, which was saturated with a mixture of O_2 + CO_2 (19 : 1) at 0° C., a 10% whole-brain homogenate (w/v) was prepared by homogenizing for 2 minutes at a speed of 14,000 r.p.m. Rat-brain homogenate from control, normothermic rats was prepared in the same way and both the homogenates were kept at 0° C. until used.

For incubation, bottle of 150 ml. capacity was used. The bottle was fitted with a rubber-based screw cap. A circular puncture was made at the centre of the cap material but not on the rubber, to facilitate injection of fluids into the flask without opening the cap and thus maintaining a closed system throughout.

Table 1

THE CONVERSION OF ^{14}C FROM (U-^{14}C) GLUCOSE AND (1-^{14}C) ACETATE INTO ^{14}CO BY CONTROL, COLD-ACCLIMATED AND HYPOTHERMIC RAT-BRAIN HOMOGENATES

	μμ moles substrate converted/mg. N	
Substrates	(U-^{14}C) Glucose	(1-^{14}C) Acetate
No. of observations	6	6
Normothermic	11360 ± 108	786 ± 18
Cold-Acclimated	12572 ± 93	604 ± 15
Hypothermic	5562 ± 41	423 ± 11

Table 2

THE INCORPORATION OF (U-^{14}C) GLUCOSE AND (1-^{14}C) ACETATE INTO FATTY ACIDS BY NORMOTHERMIC, COLD-ACCLIMATED AND HYPOTHERMIC RAT-BRAIN HOMOGENATES

	μμ moles substrate converted into fatty acids/mg. N_2 of tissues	
Substrates	(1-^{14}C) glucose	(1-^{14}C) Acetate
Number of observations	6	6
Normothermic	3.4 ± 0.6	76.7 ± 3.1
Cold-Acclimated	3.5 ± 0.3	88.5 ± 2.8
Hypothermic	2.6 ± 0.2	34.8 ± 1.2

5 ml. portions of the whole-brain homogenate from test and control rat-brain tissues were taken in separate flasks. 0.1 ml. of (1-^{14}C) acetate (5 uc) containing 125 millimicromoles of ^{14}C-acetate or 0.1 ml. of (U-^{14}C) glucose (5 μc) containing 173 millimicromoles of ^{14}C-glucose were added to each flask followed by 2.4 ml. of Krebs-Ringer phosphate buffer to give a total volume of 7.5 ml. of the incubation material. A small test-tube (1 x 7 cm.) containing 1 ml. of 10% KOH (w/v) was lowered into each flask with a pair of forceps. The gaseous atmosphere of the bottle was replaced with a mixture of O_2 + CO_2 (19 : 1) and the cap air-tightly fastened. The incubation was carried out in an Aminco Shaker for 3 hours at 37.5 ± 0.5° C. Similar incubations were carried out at 27.5 ± 0.5° C. Two ml. portions of the homogenates from control and experimental groups were taken for nitrogen determination according to the methods of Markham (4) and Felick and Munro (5).

The reaction was stopped at the end of the three-hour incubation period by injecting 0.5 ml. of 10N H_2SO_4 through the rubber cap. The bottle was shaken at 0° C: for a further 30-minute period to enable complete absorption of the $^{14}CO_2$ formed, by the KOH placed in the small tube. The tube containing KOH was removed, the bottom of the tube was rinsed with 3 ml. distilled water into the incubation bottle, the KOH containing $^{14}CO_2$ was diluted 100 times with distilled water and the radioactivity of $^{14}CO_2$ determined by using a Philips Liquid Scintillation Counter (6).

The contents of the incubation bottle was saponified by adding 3 g. solid KOH and 10 ml. absolute methanol and autoclaving for 2 hours by the method of Burchfield and Stores (7). The non-saponifiable portion was extracted five times with 25 ml. portions of petroleum ether (boiling point 40-60° C). The pooled extract was washed free of alkali with distilled water, dried with anhydrous Na_2SO_4 and the quantity of the substances extracted determined by completely evaporating the solvent and weighing the contents after keeping it overnight in vacuum desiccator. The dry residue was dissolved in 10 ml. of petroleum ether, 1 ml. taken for radioactivity determination. Another 2 ml. of the petroleum ether solution of the lipids were used for the precipitation of cholesterol as digitonide (8), a part of the cholesterol digitonide was used for radioactivity determination and the other part for chemical estimation of cholesterol by making use of the Libermann-Burchard reagent.

The contents remaining after the extraction of the non-saponifiable matter were freed completely of methanol by evaporating it on a water bath by passing a stream of air through the solution. Then it was cooled, acidified with 10N H_2SO_4 and the free fatty acids liberated were extracted with petroleum ether, and the weight determined exactly as in the case of non-saponifiable matter. The dry residue was dissolved in 10 ml. of petroleum ether, 2 ml. portions in duplicate were used for chemical estimation of free fatty acids by titrating against 0.01N NaOH and 1 ml. portion taken for radioactivity determination.

Results and discussion

The results are expressed as μμmoles of substrate (1-^{14}C) acetate are shown in table 1.

The results are expressed as moles of substrate converted into $^{14}CO_2$ by normothermic, cold acclimated and hypothermic rat-brain homogenates. It could be noted that glucose was utilized much better than acetate for conversion to $^{14}CO_2$ by a

Table 3

THE EFFECT OF COLD-ACCLIMATION AND HYPOTHERMIA ON THE INCORPORATION OF (1-^{14}C) ACETATE AND (U-^{14}C) GLUCOSE INTO THE UNSAPONIFIABLE AND DIGITONIN PRECIPITABLE FRACTIONS

	$\mu\mu$ moles of substrate converted per mg/Nitrogen			
	Unsaponifiable fraction		Digitonin precipitable fraction	
Substrate	(1-^{14}C) Acetate	(U-^{14}C) Glucose	(1-^{14}C) Acetate	(U-^{14}C) Glucose
Number of observations	6	6	6	6
Normothermic	48.1	2.3	7.6	1.3
Cold-Acclimated	56.1	2.2	8.8	1.3
Hypothermic	25.4	1.4	4.1	0.8

factor 14, 21, 11 times respectively by normothermic, cold acclimated and hypothermic rat-brain homogenates and that cold acclimated rat-brain homogenates utilized the maximum and the homogenates incubated at 27.5° C. the minimum quantity of glucose. Under hypothermic conditions, the rate of conversion of (1-^{14}C) acetate and (U-^{14}C) glucose to carbon dioxide was only about 50% when compared with that of the normothermic rat-brain homogenate at 37.5 At the same time, cold-acclimated rat-brain homogenate showed a greater ability to convert glucose to $^{14}CO_2$ as shown by the elevation of respiration by about 11%, but acetate utilisation for $^{14}CO_2$ production was down by about 23% in the same homogenate. These observations are in conformity with the manometric studies conducted earlier (9).

The extent of incorporation of ^{14}C from (U-^{14}C) glucose and (1-^{14}C) acetate into fatty acids is shown in table 2.

There was no appreciable difference in the incorporation of ^{14}C from (U-^{14}C) glucose into fatty acids in all the three types of homogenates. But radioactivity from ^{14}C-acetate was incorporated into fatty acids to a much higher extent by all the three categories of brain-homogenates when compared with the radioactivity from (U-^{14}C) glucose. The disparities in the incorporation of ^{14}C acetate into fatty acids by the three categories of rat-brain homogenates are rather conspicuous since cold-acclimated rat-brain homogenates incorporated 115% and that incubated at 27.5° only 45% of that of normothermic controls.

The incorporation of radioactivity into non-saponifiable fraction of the total lipids is shown in table 3.

The results showed that more than half the amount of glucose coverted into non-saponifiable fractions was found to be the digitonin-precipitable (cholesterol) portion, but from acetate the digitonin-precipitable fraction was about 14% only in all the three cases. At the same time, it was observed that ^{14}C-acetate was utilised 20 times better for the formation of non-saponifiable portion of the lipids as compared with (U-^{14} C) glucose. The nature of the unaccountable portion of the non-saponifiable lipids was unknown and further work is needed to elucidate the identity of this portion. There was an elevated lipogenesis from ^{14}C-acetate and increased $^{14}CO_2$ production from (U-^{14} C) glucose in the cold-acclimated rat-brain homogenate and it is possible that there may be some relationship between these two observed phenomena. With (U-^{14} C) glucose, it was observed that very little ^{14}C-activity was incorporated into the lipid fraction in all the three categories. This shows that cold acclimation or hypothermia have very little effect in **in vitro** lipogenesis from glucose by rat-brain homogenate.

A striking observation was that (1-^{14}C) acetate utilisation was at a very much higher level for fatty acids and cholesterol synthesis as compared with (U-^{14}C) glucose utilisation for the same purpose. Fatty acid synthesis, utilising acetate, is largely extra-mitochondrial (10, 11). This may account for the greater fatty acid synthesis when acetate was used as the substrate since the cells may have broken yet most of the mitochondria remaining intact.

Van Bruggen et al (12) and Srere et al (13) have observed that there was negligible incorporation of 1-^{14}C acetate into the cholesterol of the brain and spinal cord of adult rats. In young rats, however, when the body fluids were enriched with D_2O, Denterium was rapidly incorporated into the unsaponifiable fraction of the brain lipids (13, 14). The reported experiment shows that very little, if at all, of the ^{14}C-acetate was incorporated into the cholesterol molecule and thus confirms the earlier reports.

The findings that only a fraction of the total unsaponifiable portion from ^{14}C-acetate and about 50% from (U-^{14} C) glucose were digitonin-precipitable, imply that formation of other steroids or non-saponifiable materials from these substrates in the rat-brain is a possibility.

The greater conversion of ^{14}C-acetate into fatty

acids by cold-acclimated rat-brain homogenates as compared with normothermic controls may possibly be due to the enhanced activity of hexose monophosphate pathway in the brain homogenates (15) with resultant accelerated generation of $NADPH_2$, which is most essential for the synthesis of fatty acids.

Abstract

Some aspects of the incorporation of (1-^{14}C) acetate and (U-^{14}C) glucose by cold-acclimated and hypothermic rat-brain homogenates were studied and compared with those of normothermic control. There was found to be a greater incorporation of (1-^{14}C) acetate into lipid fraction compared with (U-^{14}C) glucose. But for $^{14}CO_x$production (U-^{14} C) glucose was found to be a better substrate.

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