Metabolic Changes in the Skin of Rats of Various Ages

OXYGEN CONSUMPTION AND UPTAKE OF GLUCOSE

By B. K. PATNAIK AND M. S. KANUNGO

*Physiology Laboratory, Department of Zoology, Banaras Hindu University,
Varanasi 5, India*

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1. Parallel determinations of the oxygen consumption by skin slices with glucose or galactose as substrate and of the concentration of DNA of the skin of various ages of rats were carried out to study the metabolic changes of the skin with age. 2. Both the oxygen consumption and the DNA content of the skin decreased rapidly till the attainment of maturity; thereafter the rate of decrease in oxygen consumption was lower whereas the concentration of DNA was constant. The decrease in the oxygen consumption during the early developmental period is thus due to the loss of cells, but the decrease in the older rats may be due to a decline in the activity of the cells of the skin as a result of aging. 3. The uptake of glucose by the skin decreased with increasing age.

It is well established that in certain systems of the body cellular functions decline with age (Shock, 1961). According to Barrows (1956) the overall activity of tissue slices in general, as seen from oxygen consumption and anaerobic glycolysis, declines with age. We (Kanungo & Patnaik, 1964; Patnaik & Kanungo, 1965) have shown that the uptake of ascorbic acid by the skin of the rat declines with age, which suggests a direct relationship between ascorbic acid and the synthesis of collagen by the fibroblasts of the skin. Glucose has been shown to be necessary for collagen synthesis (Flanagan & Nichols, 1964) and also as a precursor of both hexosamine and hexuronic acid (Davidson, Woodhall & Baxley, 1962). Sobel, Wright & Gabay (1958) showed that the concentration of hexosamine in the skin declines with age. In view of the above observations, oxygen consumption by skin slices with glucose and galactose as substrates and the uptake of glucose by skin slices of rats of various ages were studied to evaluate the metabolic changes in the aging rat.

MATERIALS AND METHODS

Animals. The albino rats used were of Wistar strain and were kept at $24 \pm 2^\circ$. They were fed on standard Anidiet of the Chelsea Laboratories, Poona, India. They were also given gram (*Cicer arietinum*) on alternate days and tap water *ad lib*. The rats used for the study were 4, 12, 34, 45 and 65 weeks old.

Preparation of skin slices. The animals were killed by dislocation of the neck. Several pieces of the skin from the dorsal surface were cut immediately, hairs were plucked by hand and the skin was kept in ice-cold Krebs-Ringer

phosphate-bicarbonate saline (Krebs & Henseleit, 1932) that had been oxygenated previously. Slices of the skin were weighed and then cut into thin sections with a razor blade. About 0.25 g. of skin was used for each experiment.

Oxygen consumption by skin slices. The oxygen consumption by the skin slices was measured manometrically in an Aminco refrigerated Warburg respirometer. The total incubation medium including the skin slices was 3.0 ml., which included 2.25 ml. of Krebs phosphate-bicarbonate saline, pH 7.4, and 0.5 ml. of substrate (5 mm), which was placed initially in the side arm. The centre well contained 0.2 ml. of 20% (w/v) KOH. The temperature of incubation was 37° and the gas phase was air. The skin slices were placed in the main chamber and the flasks were equilibrated by shaking at 90 oscillations/min. for 15 min. The substrate was then transferred to the main chamber and readings were taken at 10 min. intervals for 60 min. The oxygen consumption was expressed as ml. of O_2 /g. dry wt./hr. and ml. of O_2 /mg. of DNA/hr.

Glucose uptake by skin slices. The uptake of glucose by the skin slices was determined parallel with the measurement of oxygen consumption. The initial and the final concentrations of glucose in the incubation medium were determined to find out the amount of glucose taken up by the skin slices. The initial concentration of glucose was the same as that for the measurement of the oxygen consumption. Parallel controls that did not contain glucose were set up each time. Samples (1.0 ml.) of the incubation medium were withdrawn from the Warburg flasks after the 1 hr. incubation period and were mixed with equal volumes of cold 6% (w/v) trichloroacetic acid containing 0.1% (w/v) of Ag_2SO_4 . Duplicate samples of the supernatant after centrifugation were taken for the determination of the final concentration of glucose in the incubation medium according to the method of Mendel, Kemp & Myers (1954). The extinction of the colour developed was read at 520 m μ in a Beckman model DB spectrophotometer. The concentrations of glucose in the unknown samples

were determined from a standard curve which was linear and was drawn each time. The uptake of glucose was expressed as mg. of glucose/g. wet wt./hr. and mg. of glucose/g. dry wt./hr.

DNA content of the skin. Pieces of the skin from the dorsal surfaces of 1-day-old and 4-, 12-, 45- and 65-week-old rats were cut and the hairs were removed. Portions (1.0g.) of skin were ground in 10.0ml. of water in a precooled mortar containing a small amount of chemically pure sand. Then 5.0ml. of 10% (w/v) trichloroacetic acid was added to 2.0ml. of the resulting homogenate. The mixture was centrifuged at 6000g_{av.} at 0° for 10 min. in an International refrigerated centrifuge. The precipitate was resuspended in 5.0ml. of 10% trichloroacetic acid and centrifuged at 6000g_{av.} for 10 min. This precipitate was extracted twice with 10ml. of 90% (v/v) ethanol and was then suspended in 1.3 ml. of water. The rest of the procedure for the determination of the concentration of DNA was the same as that of Schneider (1957). The extinction of the colour developed was measured at 600m μ . The calculations were based on the datum that 1.0 μ g. of DNA gives an extinction of 0.019 at 600m μ . The concentration of DNA was expressed as mg. of DNA/100g. dry wt. of skin.

Standard deviations of the mean and the levels of significance were calculated according to the method of Garret (1956).

RESULTS

Figs. 1 and 2 show that the oxygen consumption of the skin slices with glucose or galactose as the substrate declined sharply till 12 weeks of age, both

Fig. 1. Oxygen consumption of the skin of 4-, 12-, 34-, 45- and 65-week-old rats. Slices were incubated as described in the text. ●, With D-galactose as substrate (4-, 12- and 65-week-old female rats); ○, with D-glucose as substrate (4-, 12-, 34-, 45- and 65-week-old male rats). Each point represents the mean of seven experiments and the vertical bars represent $\pm$ S.D.

on the basis of dry weight and DNA content of the skin. Thereafter the oxygen consumption decreased slowly until 65 weeks of age. The differences between the oxygen consumption by the skin of various age groups were highly significant.

Fig. 3 shows that the uptake of glucose by the skin slices decreased with age. The differences between the uptake by the skin of various age

Fig. 2. Oxygen consumption by the skin of rats. Experimental details are given in the text. ●, With D-galactose as substrate (4-, 12- and 65-week-old female rats); ○, with D-glucose as substrate (4-, 12-, 34-, 45- and 65-week-old male rats). Each point represents the mean of seven experiments and the vertical bars represent $\pm$ S.D.

Fig. 3. Uptake of glucose by the skin slices of 4-, 12- and 45-week-old male rats. Experimental details are given in the text. ●, mg. of glucose/g. dry wt./hr.; ○, mg. of glucose/g. wet wt./hr. Each point represents the mean of seven experiments and the vertical bars represent $\pm$ S.D.

Table 1. *Concentration of DNA in the skin of rats of various ages*

Determinations were made as described in the text. Results are given as means $\pm$ S.E.M., with the numbers of animals in parentheses.

Age (days)	Concn. of DNA (mg./100g. dry wt.)	Significance of change (P)
1	69.19 $\pm$ 4.07 (3)	< 0.001
28 (4 weeks)	23.02 $\pm$ 1.23 (10)	
84 (12 weeks)	18.27 $\pm$ 0.09 (4)	< 0.01
315 (45 weeks)	17.16 $\pm$ 0.54 (4)	
455 (65 weeks)	15.49 $\pm$ 0.39 (6)	0.05

groups were highly significant. Table 1 shows that the concentration of DNA in the skin declined rapidly during the growth period. The concentrations of DNA in the skin of 12- and 45-week-old rats were not significantly different. The DNA content of the skin of 65-week-old rats was slightly lower than those of 12- and 45-week-old rats.

DISCUSSION

The present studies show that oxygen consumption by the skin slices with glucose or galactose as the substrate is highest in the 4-week-old rats but declines rapidly as the animals reach maturity (12 weeks old). Thereafter the oxygen consumption decreases with increasing age (Figs. 1 and 2). Simultaneous determination of the DNA content of the skin also shows a rapid decline during the early development of the rat till it becomes 12 weeks old. After this the decline is insignificant (Table 1). Therefore the oxygen consumption of the skin in the early developmental period at least appears to be related to cell number. However, in the older rats this may not be so. Though there is no difference in the cell number between the skins of 12- and 45-week-old rats on the basis of DNA content, the oxygen consumption of skin of the older rats is significantly lower ($P < 0.001$) than that of the younger rats. The lower oxygen consumption by skin of the 45-week-old rats may thus be due to the decreased activity of the cells of the skin as a result of aging.

The oxidative system of the cells is impaired in ascorbic acid deficiency (King, 1950) and the concentration of ascorbic acid in the skin of rats declines with age (Kanungo & Patnaik, 1964). It is possible therefore that the lowering of concentration of ascorbic acid in the skin of rats may be responsible in some way for the decreasing oxygen consumption with age.

The uptake of glucose by the skin slices of rat declines with age (Fig. 3). Glucose is necessary for

the synthesis of collagen in that the carbon skeletons of the amino acids are derived from it (Flanagan & Nichols, 1964). The higher uptake of glucose by the skin of 4-week-old rats as compared with that of older rats may be due to two reasons: (1) since the rate of collagen synthesis in the skin of rats remains high till the age of 5 weeks (Wirtschafter & Bentley, 1962), the demand for glucose may also be high during this period; (2) the fibroblasts may be metabolically more active during the rapid synthesis of collagen in the young rats and may require more energy, which is derived by the oxidation of glucose.

Glucose has also been shown to be incorporated into the mucopolysaccharides of cultivated connective tissue (Guha, Mukherjee, Balasubramanian & Bacchawat, 1964). Davidson *et al.* (1962) found that glucose serves as a precursor of glucosamine. Further, the concentration of hexosamine, which is a component of the mucopolysaccharide of the skin, declines with age (Sobel *et al.* 1958). Hence the decrease in the uptake of glucose by the skin may also be due to a lower rate of synthesis of hexosamine, which may affect the synthesis of mucopolysaccharide.

In most of the tissues studied by Shock (1960) and Barrows, Roeder & Falzone (1962) no significant change in the DNA content was found when young and adult rats were compared. Nikitin, Novikova & Zikalo (1960) determined the DNA content of the heart, kidney, intestine and liver of newborn and 1-, 3-, 12- and 24-month-old rats, and found that its concentration decreased till the age of 3 months, after which there was no change. Our results show that also in the skin the DNA content declines till the age of 12 weeks, after which there is no significant decrease (Table 1). This shows that cell loss occurs only during the early developmental period. After the attainment of maturity the rate of cell loss is insignificant. However, since the skin continues to expand even after maturity, this increase in the surface area may be due to increasing accumulation of collagen fibres and mucopolysaccharides in the intercellular spaces of the skin.

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