skulls were deposited in the collection of the Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo. Air-dried preparations of bone marrow and testis were made after in vivo colchicine treatment. G- and C-banding were performed according to routine techniques[8,9]. Silver staining followed the method described by Lau et al.[10].

All specimens had a diploid number of 2n = 34, with an autosomal complement of 14 metacentric or submetacentric pairs and 2 small acrocentric pairs. The pair 1 presents a large distal secondary constriction in the long arm. The X is a medium sized acrocentric and the Y a small one, both perfectly identifiable morphologically (fig. 1). This karyotype has previously been described by Yonenaga[6].

About 360 metaphases were analyzed using banding techniques. G-band patterns of a female karyotype are shown in figure 2. The C-banded karyotype presents heterochromatic blocks at the centromeres of the autosomes, with the exception of pairs 2, 3 and 4 which show no heterochromatin. The X chromosome, corresponding to about 8% of the haploid set, has heterochromatin in the centromere region and in the proximal segment corresponding to one third of its long arm. The Y chromosome, whose relative size is about 4.5% of the haploid set, is entirely heterochromatic (fig. 3). The relatively large size of the sexual pair appears to be due to the presence of large amounts of constitutive heterochromatin. In *Clyomys laticeps laticeps*, NORs are restricted to the secondary constriction of pair 1 (fig. 4). In all 62 cells analyzed, 2 pairs of Ag-NORs per methaphase were always found and these homologues were never seen in association. The presence of a large secondary constriction in only 1 pair of autosomes is characteristic of the family Echimyidae; the morphology and size of this chromosome pair is, however, variable. In the species where silver staining was used, *Trichomys apereoides, Proechimys iheringi iheringi* and *Clyomys laticeps laticeps*, the chromosome pair with secondary constriction is indeed the NOR chromosome.

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3 Cabrera, A., Reota Mus. argent. Cienc. nat. *6* (1961) 353.
4 Reig, O.A., Barros, M.A., Useche, M., Aguilera, M., and Linares, O.J., Genetica *51* (1979) 153.
5 Reig, O.A., Aguilera, M., Barros, M.A., and Useche, M., Genetica *52/53* (1980) 291.
6 Yonenaga, Y., Caryologia *28* (1975) 269.
7 Souza, M.J., and Yonenaga-Yassuda, Y., Cytogenet. Cell Genet. *33* (1982) 197.
8 Seabright, M., Lancet *2* (1971) 971.
9 Sumner, A.T., Exp. Cell Res. *75* (1972) 304.
10 Lau, Y.F., Pfeiffer, R.A., Arrighi, F.E., and Hsu, T.C., Am. J. human Genet. *30* (1978) 76.

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Age-related response of citrate synthase to hydrocortisone in the liver and brain of male rats[1]

R. Sharma[2] and S.K. Patnaik[3]

Biochemistry Division, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi-221005 (India), 16 April 1982

Summary. The activity of citrate synthase of the liver and brain of rats shows a gradual increase as a function of age. Adrenalectomy causes no significant change in the activity of citrate synthase in either of these tissues in young, adult or old rats. Administration of hydrocortisone to adrenalectomized rats depresses the activity of this enzyme maximally in the liver and brain of young rats. Administration of actinomycin D tends to normalize the depressed level of this enzyme.

Citrate synthase (citrate oxaloacetate lyase (CoA-acetylating) EC 4.1.3.7) is the first enzyme of the Krebs cycle, which catalyzes the conversion of oxaloacetic acid and acetyl-CoA to citric acid, a step which is considered to be the major regulatory site of Krebs cycle activity[4]. There are only a few conditions known to change the total activity of citrate synthase in rat tissues. Prolonged exercise causes a 2-fold increase in the activity of muscle citrate synthase[5]. Kirsten and Kirsten[6] have demonstrated that aldosterone injections cause a transient 30% increase in the level of citrate synthase. It has also been reported that administration of dexamethasone increases and decreases, respectively, the activity of PEPCK and citrate synthase in rat liver[7]. Mukherjee et al.[8] reported that the activity of hepatic citrate synthase increases by 2-3-fold in vitamin B_{12} deficiency. In the present investigation, citrate synthase was selected as a model enzyme, since it is the key regulatory enzyme of the Krebs cycle, and the rate of oxidation through the Krebs cycle might be controlled by the rate of citrate synthase activity as limited by oxaloacetate concentration. There has been no report so far on any enzyme that is depressed and repressed by hydrocortisone and actinomycin D, respectively, in any organism during different phases of its life-span.

Materials and methods. Male albino rats of the Wistar strain of 3 different age groups (6-, 30- and 90-week-old), maintained under standard laboratory conditions, were used.

Pilot experiments were undertaken to investigate the time and dose dependence of citrate synthase in rats of various ages given hydrocortisone. The rats of each age group were divided into 4 sets with 4–5 rats each. The set-1 rats served as the control. The rats of sets 2, 3 and 4 were bilaterally adrenalectomized. These rats were given 0.9% NaCl ad libitum instead of water for 10 days following adrenalectomy. On the 11th day, the set-2 rats received 1.0 ml of 0.9% NaCl i.p. and these rats served as the control for the induction studies. The rats belonging to sets 3 and 4 were given an i.p. dose of hydrocortisone (5.0 mg/100 g b.wt, suspended in 1.0 ml of 0.9% NaCl) at a fixed time of day for 3 days. The set-4 rats were also given actinomycin D (10.0 µg/100 g b.wt, suspended in 1.0 ml of 0.9% NaCl), 1 h prior to the hydrocortisone administration, for 3 days. All the rats were killed 3 h after the final injection.

The rats were killed by cervical dislocation, and their livers and brain tissues were taken out. The mitochondria were separated[9] and the activity of citrate synthase was determined by measuring the initial rate of the reaction at 412 nm by the DTNB method[10]. One unit of this enzyme is the amount that catalyzes the liberation of 1 µmole of CoA-SH/min under the standard conditions. The activity

Effects of adrenalectomy (A/d), hydrocortisone (HC) and actinomycin D (A) on the activity (units/mg protein) × 10^3 of citrate synthase of the liver and brain of male rats of various ages

Tissue	Treatments	6-week-old Mean SD	p	30-week-old Mean SD	p	90-week-old Mean SD	p
Liver	Normal	25.40 ± 0.70	NS	29.60 ± 0.83	NS	33.50 ± 1.10	NS
	A/d	26.60 ± 1.70 (NE)	< 0.01	30.50 ± 1.50 (NE)	< 0.01	39.20 ± 1.20 (NE)	
	A/d + HC	18.00 ± 1.50 (− 33%)	< 0.01	23.00 ± 0.90 (− 25%)	< 0.01	39.70 ± 1.10 (NE)	NS
	A/d + A + HC	26.10 ± 2.10 (+ 45%)		27.10 ± 0.98 (+ 17%)		38.50 ± 1.50 (NE)	
Brain	Normal	137.30 ± 12.50	NS	175.30 ± 11.90	NS	199.10 ± 8.50	NS
	A/d	137.60 ± 2.90 (NE)	< 0.001	193.30 ± 10.20 (NE)	NS	184.20 ± 11.00 (NE)	NS
	A/d + HC	110.40 ± 2.40 (− 20%)	NS	174.40 ± 12.00 (NE)	NS	183.30 ± 10.30 (NE)	NS
	A/d + A + HC	119.50 ± 7.00 (NE)		163.80 ± 10.20 (NE)		194.00 ± 6.70 (NE)	

The data were collected from 4–5 rats of each age group. Standard deviation (SD) and the levels of significance ($p < 0.05$) are given. +, increase; −, decrease; NE, no effect; NS, not significant.

of the enzyme is expressed as units/mg protein. Protein content of the mitochondrial suspensions was determined[11]. All the data were statistically analyzed[12].

Results and discussion. Our data indicate that the activity of citrate synthase shows a gradual increase in the liver and brain as a function of the age of the rats (table). Thus the slight increase in the activity of this key Krebs cycle enzyme may make these tissues more aerobic during this phase of the life-span. These findings are consistent with earlier findings on lactate dehydrogenase[13] and mitochondrial malate dehydrogenase[8]. Land et al.[14] have also reported that the activity of citrate synthase in the brain of the adult rat is much higher than that in the immature rat. The basal level of this enzyme is also significantly higher in the brain as compared to that of the liver in rats of all ages which clearly indicates the high oxidative capacity of the brain as compared to that of the liver since the amount of citrate synthase in the cell is directly correlated with the ability of the cell to utilize oxygen[15].

Adrenalectomy (A/d) caused no significant effect on the activity of citrate synthase of the liver and brain of rats of any age. This shows that the activity of citrate synthase of these tissues is not much dependent on the adrenal gland of the animal[16]. Administration of hydrocortisone to A/d rats caused a depression in the activity of this enzyme in the liver of young and adult rats but not in the old rats. However, in the brain this hormone depressed the activity of citrate synthase in the young rats only. Administration of actinomycin D prior to the hydrocortisone treatment tends to normalize the depressed level of this enzyme following hydrocortisone treatment. Thus the findings provide a firm support for the general hypothesis[17] that oxaloacetate, an intermediate of gluconeogenesis, is diverted from the TCA cycle to gluconeogenesis, owing to the higher level of the enzyme which converts it to phosphoenolpyruvate, thereby diminishing the rate of this cycle. Our present observations along with the earlier findings of Heitzman et al.[7] confirm the above-mentioned hypothesis that increase in the rate of gluconeogenesis following glucocorticoid administration is related to an increase and decrease respectively in the activity of cytoplasmic phosphoenolpyruvate carboxykinase (PEPCK) and citrate synthase. Further, the present observations are better clarified by the findings that actinomycin D treatment tends to normalize the depressed level of citrate synthase in the liver of young and adult rats, possibly by inhibiting the induction of PEPCK by hydrocortisone[18]. However, when the brain PEPCK is not very much affected by hydrocortisone, the level of citrate synthase remains almost unaffected. It should also be mentioned here that the degree of the reactions to hydrocortisone administration, i.e. induction of PEPCK and depression of citrate synthase, decreases in the liver as a function of the age of the rats.

On the basis of the present findings, it may be concluded that oxaloacetic acid is diverted more towards gluconeogenesis after the administration of hydrocortisone, and it is used for the synthesis of glucose. These findings, however, lead us to the conclusion that alterations in the levels and the differential regulation of enzymes by various endogenous factors (such as hormones) may account for the development and aging of an organism[18].

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2 Department of Life Sciences, Manipur University, Canchipur, Imphal 795003/India.

3 To whom reprint requests should be addressed.

4 Srere, P.A., in: Current Topics in Cellular Regulation, vol. 5, p. 229. Eds B.L. Horecker and E.R. Stadtman. Academic Press, New York 1972.

5 Holloszi, J.O., Oscai, L.B., Don, I.J., and Mole, P.A., Biochem. biophys. Res. Commun. *40* (1970) 1368.

6 Kirsten, R., and Kirsten, E., Am. J. Physiol. *223* (1972) 229.

7 Heitzman, R.J., Herriman, I.D., and Malinson, C.B., FEBS Lett. *20* (1972) 19.

8 Mukherjee, A., Srere, P.A., and Frenkel, E.P., J. biol. Chem. *251* (1976) 2155.

9 Sharma, R., and Patnaik, S.K., Devl. Growth Different. *24* (1982) 501.

10 Srere, P.A., Brazil, H., and Gonen, L., Acta chem. scand. *17* Suppl. (1963) 129.

11 Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., J. biol. Chem. *193* (1951) 265.

12 Garrett, H.E., Statistics in Psychology and Education. David McKay Company Inc., New York 1966.

13 Singh, S.N., and Kanungo, M.S., J. biol. Chem. *243* (1968) 4526.

14 Land, J.M., Booth, R.F.G., Berger, R., and Clark, J.B., Biochem. J. *164* (1977) 667.

15 Srere, P.A., Biochem. Med. *3* (1969) 61.

16 Law, P.Y., and Edelman, I.S., J. Membrane Biol. *41* (1978) 41.

17 Krebs, H.A., in: Advances Enzyme Regulation, vol. 4, p. 339. Ed. G. Weber. Pergamon Press, Oxford 1966.

18 Sharma, R., and Patnaik, S.K., Indian J. Biochem. Biophys. *18*, suppl. (1981) 73.

19 Kanungo, M.S., Biochemistry of Aging. Academic Press, London 1980.

0014-4754/84/010097-02$1.50 + 0.20/0