The Independence of Cell Division and Age-Dependent Modification of Enzyme Induction

Richard C. Adelman

Fels Research Institute and Department of Biochemistry,
Temple University Medical School, Phila., Pa. 19140

Received February 13, 1970

SUMMARY

The age-dependent modification of hepatic glucokinase inducibility was employed as a parameter of biological aging in the rat. Synchronous division of liver cells was induced by partial hepatectomy. Fully regenerated liver retained the pattern of enzyme inducibility characteristic of the untreated animal of the same age. The age-dependent modification of enzyme inducibility in rat liver is unrelated to cell division, and may reflect an aging phenomenon of extrahepatic origin.

An aging organism may be characterized, in part, by its reduced capacity to adapt to environmental change. Adaptive responses are reflected at the molecular level by modified rates of protein biosynthesis and degradation, as well as by altered physiological activities. Consequently, it is not surprising that the susceptibility of certain enzymes to nutritional, hormonal and pharmacological regulation varies with age (1-9).

An age-dependent lag period in the induction of rat liver glucokinase by glucose-feeding recently was demonstrated in this laboratory (1). In order to gain more insight into the relationship of this age-dependent enzyme induction to cell growth, the kinetics of the glucokinase induction were examined as a function of age in fully regenerated rat livers following partial hepatectomy.

EXPERIMENTAL PROCEDURE

Animals - Young, normal male rats of the Sprague-Dawley strain, purchased from Carworth Farms, were maintained on a commercial stock diet and were used at 2 to 10 months of age. Old, normal male rats of the same strain were obtained as retired breeders from the National Cancer Institute (CR: RAR-SD), and were maintained on the same commercial stock diet. They were used when they attained the ages indicated in the text.

Partial hepatectomy was performed according to the method described by Higgins and Anderson (10).

Chemicals and Materials - All purified enzymes and other biochemical reagents were purchased from the Boehringer-Mannheim Corporation.

Dietary Treatment - Following a 72-hour fast rats were refed by intragastric injection of 5 mmoles of glucose per 200 g of body weight, and then fed ad libitum, until the indicated time of sacrifice, the high-glucose diet described previously (1).

Tissue for Enzyme Assay - Rats were decapitated and livers were dissected, cleaned, weighed and homogenized with one volume of cold 10 mM Tris-HCl-1.0 mM EDTA, pH 7.5, in a glass homogenizer with a Teflon pestle. The homogenate was centrifuged for one hour at 105,000 x g in a Spinco model L ultracentrifuge at 2^{o}. Glucokinase was assumed to be entirely in the high speed supernatant (11).

Assay Method - Glucokinase activity was assayed at 25^{o} with a Gilford model 2400 recording spectrophotometer, according to a well-established procedure (12). The conditions for this procedure were verified for the various nutritional manipulations of each age group of treated and untreated rats.

RESULTS AND DISCUSSION

The present series of experiments were designed to determine whether

newly created rat liver cells, within an aged animal, possess the biochemical properties of young or old rat liver. Synchronous division of liver cells was induced in vivo by partial hepatectomy in groups of rats ranging in age from 2 months to 2 years. The kinetic pattern of the glucokinase induction was employed as a parameter of biological aging (1). As illustrated in Figure 1a-d, the fully regenerated liver retains the

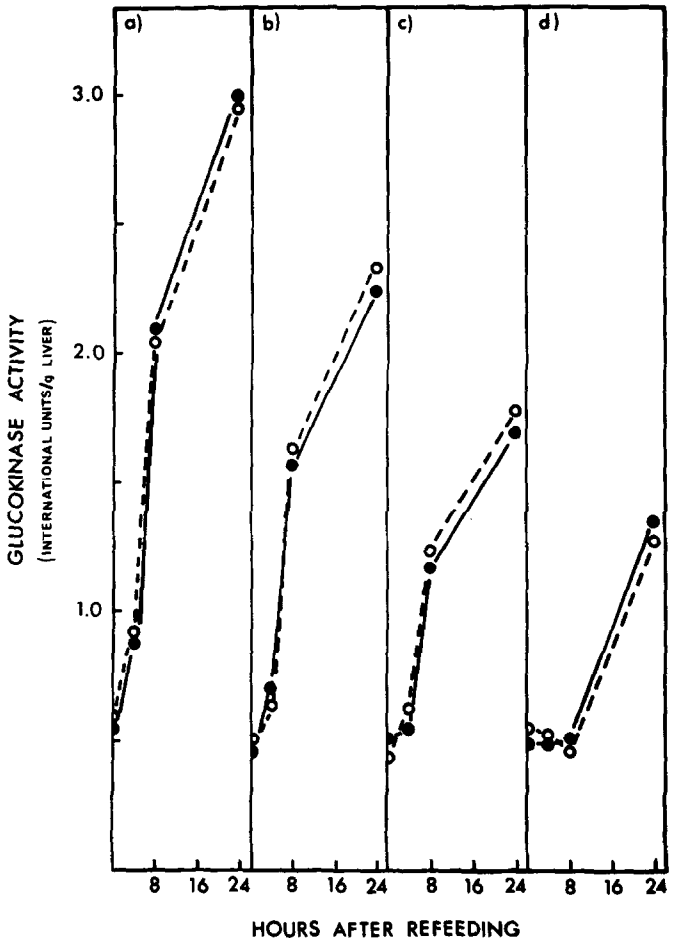

Figure 1. The effect of synchronous liver cell division on the age-dependent inducibility of glucokinase. Partial hepatectomy and nutritional manipulations were performed as described in the Experimental Procedure. Glucokinase activity was assayed at the time intervals indicated in the Figure. The ages of the groups of rats employed were a) 2 months, b) 10 months, c) 18 months and d) 24 months. ●——● represents untreated rats and O- - -O partially hepatectomized rats in each age group. Each point represents the average value of at least four rats.

pattern of glucokinase inducibility characteristic of the untreated animal of the same age. Although the data are not shown, the initial fed values of glucokinase activity, about 2.5 international units per g of liver weight, always were completely restored in no more than 48 to 72 hours. Statistical analysis of the age-dependent modification of the glucokinase induction was presented previously (1).

Hayflick and Moorhead (13) have shown that the cell population of normal human diploid fibroblasts of embryonic origin is capable of doubling *in vitro* only 40 to 60 times. The embryonic cells then cease to divide, and the cell population dies. This phenomenon is utilized as an *in vitro* model of biological aging, and is attributed to an accumulation of errors in DNA molecules and, eventually, to errors in protein synthesis (14).

It is apparent from the data of the present report that cellular aging phenomena, *in vivo*, may be completely unrelated to the process of cell division. Furthermore, in a recent demonstration of an age-dependent lag period in the induction of mouse liver tyrosine transaminase by exposure to cold, Finch *et al.* (2) concluded that the potential for gene-mediated cellular response is not impaired by aging. Their data, as well as the data presented in this report and also in a previous one (1), are consistent with the age-dependent formation of either a modified protein, or a distinct new protein, whose catalytic properties are such that the observed lag periods result during the inductive process. Although the accumulation of errors in cellular information machinery (15) is a fashionable hypothesis that has been employed by many investigators to account for biological aging, there is no discrete experimental evidence, i.e., the isolation of modified protein, in support of such phenomena.

Therefore it is tempting to speculate that the age-dependent modification of enzyme induction in rat liver reflects an aging phenomenon of extrahepatic origin. The immediate cause, then, must be attributed to either humoral or neural events, whose potential importance already have been discussed (1,2).

Experiments to localize the origin of age-dependent changes in hepatic enzyme induction are underway in this laboratory.

ACKNOWLEDGEMENTS

The author gratefully acknowledges Miss Kathleen Blasiak for her excellent technical assistance. This work was aided by Grants CA 10439 and CA 10916 from the National Institutes of Health, and Grant P 202 F from the American Cancer Society.

REFERENCES

1. Adelman, R.C., J. Biol. Chem., 245, (1970).
2. Finch, C.E., Foster, J.R., and Mirsky, A.E., J. Gen. Physiol., 54, 690 (1969).
3. Haining, J.L., and Correll, W.W., Arch. Biochem. Biophys., 117, 266 (1966).
4. Correll, W.W., Turner, M.D., and Haining, J.L., J. Gerontol., 20, 507 (1965).
5. Haining, J.L., and Correll, W.W., J. Gerontol., 24, 143 (1969).
6. Singhal, R.L., J. Gerontol. 22, 77 (1967).
7. Singhal, R.L., J. Gerontol. 22, 343 (1967).
8. Houck, J.C., and Jacob, R.A., Proc. Soc. Exptl. Biol. Med., 113, 692 (1963).
9. Kato, R., and Takanaka, A., J. Biochem., 63, 406 (1968).
10. Higgins, G.M., and Anderson, R.M., Arch. Pathol., 12, 186 (1931).
11. Shatton, J.B., Morris, H.P., and Weinhouse, S., Cancer Research, 29, 1161 (1969).
12. Sharma, C., Manjeshwar, R., and Weinhouse, S., J. Biol. Chem., 238, 3840 (1963).
13. Hayflick, L., and Moorhead, P.S., Exp. Cell Res., 25, 585 (1961).
14. Hayflick, L., Exp. Cell Res., 37, 614 (1965).
15. Orgel, L.E., Proc. Nat. Acad. Sci., U.S., 49, 517 (1963).