AGE-DEPENDANT VARIATION IN RIBONUCLEIC ACID CONTENT OF THE RAT DIAPHRAGM*

J. S. BAJAJ AND D. D. BANSAL

Department of Medicine,
All-India Institute of Medical Sciences New Delhi

In vitro preparation of the rat diaphragm has been extensively used for studying the action of insulin on certain metabolic parameters such as glucose uptake (4), glycogen synthesis (13), protein synthesis (5) and ribonucleic acid (RNA) synthesis (14, 15). While there is a general agreement that insulin stimulates the first three of these parameters, its effect on the RNA synthesis is controversial (6, 14, 15). Thus, insulin stimulated RNA synthesis demonstrated by some workers (14, 15) has not been substantiated by others (6). The reason for these conflicting results is not known. The RNA content, RNase (nuclease) activity and RNA synthesis in the rat liver varies with the age of the animal (2, 9). This observation prompted us to study the RNA content of the rat diaphragm in two different age groups of rats.

MATERIALS AND METHODS

Male albino rats (Wistar strain - maintained at this Institute) of two different groups were used. The 4-5 week old animals weighed 50-60 *g* and the 12-14 week old, 120-130 *g*. They were starved for 24 hr and then killed by cervical dislocation and decapitation. The diaphragm was quickly excised from the surrounding thoracic cage and was dissected clean. The thick central dorsal tendon was excluded and the remaining diaphragm was divided into two approximately equal right and left halves (hemidiaphragms). Schneiders' technique (11) of RNA extraction was followd. Each hemidiaphragm was weighed, placed in ice-chilled 10% W/V trichloroacetic acid (TCA) in Petri dishes and was finely cut. It was homogenized in the tissue homogenizer with adequate volume of cold TCA. After homogenization, the contents were centrifuged at 1500 rpm for 6 min. The supernatant was discarded and the residue was washed twice with 5 *ml* 5% TCA, each time discarding the supernatant after centrifugation at 1500 rmp for 6 min. The residue was then washed successively with 95% V/V alcohol, alcohol ether mixture (1:3 V/V) and ether. The resultant residue when mixed with 2.4 *ml* TCA and heated at 80°C for 15 min, yielded a clear solution which was used for RNA estimation for which Mejbaum's modified method (8) based on the orcinol reaction was followed. Aliquot of 0.6 *ml* of the individual extracts was diluted to 3 *ml* by

*Received 22-4-1970.

distilled water. To this was added 3 *ml* of 1% orcinol in 0.1% ferric chloride i (36.4% W/W) hydrochloric acid. The tubes were then heated at 100°C for 20 min in a boilin water bath. After the contents had cooled, RNA was determined by measuring the absorp tions at 660 *mμ*. The RNA concentration in the extracts was calculated from the standar graph obtained from the known dilutions of yeast RNA (S.V.P. Chest Institute, Delhi-7).

RESULTS

The results are presented in Table I.

TABLE I

RNA content of hemidiaphragm tissue in rats of two age groups.

Rats		*Mean wet weight (mg) of the hemidiaphragm per rat ±S.E.*	*weight (mg) of the hemidiaphragm expressed per 100 g body weight*	*Mean RNA (mg) per hemidiaphragm ±S.E.*	*Mean RNA (μg) per 100 mg of the hemidiaghragm ±S.E.*
Age weeks	*Weight G.*				
4-5	50-60	43.8 ±5.16	79.5	142.8 ±3.92	325.5* ±4.48 (16)
12-14	120-130	95.8 ±13.64	76.6	208.8 ±8.4	217.2* ±4.48 (16)

*P<0.001 by t-test. Figure in parentheses indicate the number of hemidiaphragms.

The concentration of RNA in the hemidiaphragm tissue was 325.5 *μg*/100 *mg* wet weight in the 50-60 *g* weight rats and 217.2 *μg*/100 *mg* wet weight in the 120-130 weight rats. The difference was very highly significant (P<0.001). The average RNA level per hemidiaphragm is also given in the Table.

DISCUSSION

The effect of age on the RNA content of brain (2), liver (2), intestine (10) in the rat, biceps femoris and semimembranosus muscle in the pig (3) and spinal cord in the man (12) has been reported. In all these studied a decrease in the RNA content was noticed with advancing age. However, the effect of age on the RNA content of rat diaphragm muscle does not seem to have been studied. Matsumoto and Krasnow (7) reported that RNA contents of the diaphragm of adult rats (150-580 *g*) was 115 *μg*/100 *mg* wet weight of the diaphragm. Their observation is in agreement with the conclusion of the present study that there seems to be a progressive reduction in the RNA content of the diaphragm with the advancing age. The protein content of the diaphragm in these two groups of rats was found to be nearly identical.

Thus the significant difference in their RNA concentration cannot be attributed to factors such as tissue water content.

SUMMARY

RNA concentration in the hemidiaphragm tissue was significantly less in the 12-14 week old rats than in the 4-5 week old rats.

ACKNOWLEDGEMENTS

It is a pleasure to thank Dr. K. Ramachandran, Assistant Professor of Statistics and Dr. D. N. Sharma of the Central Animal House of this Institute for their generous help and, Dr. N. K. Bhide, for help in the preparation of manuscript.

REFERENCES

1. Bajaj, J.S. and D.D. Bansal. DNA and protein content of rat diaphragm tissue in the various age groups of rats (*to be published*).
2. Devi, A., M.A. Mukudan, U. Srivastava and N.K. Sarkar. The effect of age on the variation of deoxyribonucleic acid. Ribonucleic acid and total nucleotides in liver, brain and muscles of rat. *Experimental Cell Res*, **32** : 242, 1963.
3. Durand, G., F. Guy and P. Eliane. Content of nucleic acid and protein in pig muscle in postnatal growth. *C.R. Acad. Sci. (D) (Paris)*, **264** : 1640, 1967.
4. Gemill C.L. Insulin on the metabolism of the isolated diaphragm of the rat. *Bull. John Hopk. Hosp.*, **68** : 329, 1941.
5. Krahl, M.E. Incorporation of C^{14} amino acids into peptides by normal and diabetic rat tissues. *Science*, **116** : 524, 1952.
6. Manchester, K.L. Re-evaluation of the effect of insulin on nucleic acid synthesis in muscle. *Biochem J.*, **105** : 13C, 1967.
7. Matsumoto, S. and N. Kransons. Nucleic acid metabolism of cardiac, diaphragmatic and quadriceps muscle. *Am. J. Physiol.*, **214** : 620, 1968.
8. Mejbaum, W.J. Estimation of small amounts of pentose especially in derivatives of adenylic acid. *Z. Physiol. Chemic.*, **258** : 117, 1939.
9. Mukudan, M.A., A. Devi and N.K. Sarkar. The effect of age on the variation of nuclease activities and other possible function in rat liver. *Experimental Cell Res.*, **32** : 251, 1963.
10. Quarto Di Palo, F.M., L.E. Mombelli and L. Gastaldi. Age changes in phospolipids, RNA, DNA contents in tissues of male white rats. *Gerontologia*, **10** : 161, 1964.
11. Schneider, W.C. Phosphorus compounds in animal tissues. Extraction and estimation of deoxypentose nucleic acid and of pentosenucleic acid. *J. Biol. Chem.* **161** : 293, 1945.

12. Seize, K. and V. Thierbach. Age changes and biochemistry of human spinal cord. Nucleic acid content at various ages. *Z. Alternsforsch.*, **16** : 211, 1963.

13. Stadie, W.C. and J.R. Zapp. The effect of insulin upon the synthesis of glycogen by rat diaphragm *in vitro*. *J. Biol. Chem.*, **170** : 55, 1947.

14. Wool, I.G. Insulin and incorporation of radioactivity into nucleic acid fraction of isolated diaphragm. *Am. J. Physiol.* **199** : 719, 1960.

15. Wool, I.G. Effect of insulin on nucleic acid synthesis in isolated rate diaphragm. *Biochem. Biophys. Acta.*, **68** : 28, 1963.