COMPARATIVE STUDIES OF THE AGE-RELATED CHANGES IN PROTEIN SYNTHESIS IN THE RAT PANCREAS AND PAROTID GLAND

S. K. KIM, P. A. WEINHOLD, D. W. CALKINS and V. W. HARTOG

Research Service, VA Medical Center, Ann Arbor, MI 48105, U.S.A. and
The University of Michigan Medical School

INTRODUCTION

PREVIOUS studies have reported that, as humans age, changes occur in the secretory function of the exocrine digestive glands, such as the pancreas and salivary glands. In the pancreas, a reduction in secretion has been reported in aged individuals (Necheles *et al.,* 1942). Apparently, there is no detectable change in the pancreatic stimulation by secretin (Rosenberg *et al.,* 1966). Upon repeated stimulations with pancreozymin and secretin, however, the secretion of amylase decreases in older persons, although the secretion of bicarbonate does not change (Bartos and Groh, 1969).

In the case of the salivary gland, the rate of salivary flow, basal as well as the stimulated secretion of saliva, is reduced in elderly persons (Meyer and Necheles, 1940; Bertram, 1967). Also, the enzyme content of the saliva decreases with increasing age; a reduction in the content of amylase in mixed saliva of elderly persons has been reported (Meyer *et al.,* 1937; Meyer and Necheles, 1940; Chilla *et al.,* 1974).

These reductions in the content and secretion of digestive enzymes suggest that the cellular level of the enzymes is reduced with aging, possibly due to the decline in the synthesis of secretory proteins. A reduction in protein synthesis with age has been demonstrated in many animal tissues such as the prostate gland (Mainwaring, 1968), liver (Mainwaring, 1969; Hrachovec, 1969; Kurtz, 1975; Layman *et al.,* 1976), brain (Murthy, 1966; Johnson, 1968; Johnson and Belytschko, 1969), muscle (Breuer *et al.,* 1964; Srivastava, 1969), testis (Liu *et al.,* 1978) and lens (Klethi, 1976).

We have shown previously that the cellular level of protein synthesis declines with age in the rat parotid salivary gland (Kim *et al.,* 1979; 1980; Kim, 1981). In this study we have examined the changes in the cellular level of a digestive enzyme, α-amylase, and the synthesis of protein in the rat pancreas. The results from this study have been compared with those changes detected in the parotid gland.

MATERIALS AND METHODS

Animals

Male, Sprague–Dawley rats of 2, 12, 18, 24 and 30 months of age were used and obtained from Charles River Breeding Laboratories in Wilmington, Massachusetts. All the rats were of the same strain of Caesarean derived rats, except that the older ones (12 months and older) came from the aging colony maintained by the N.I.A. at the above breeding laboratory. All rats were housed in an isolator upon arrival and allowed to acclimate for several days before beginning experiments. Food was removed from these rats about 17–18 h before the experiment, but they were allowed access to drinking water. All the rats were killed between 8 and 9 a.m. on the day of the experiments by cervical dislocation. The pancreas and parotid glands were removed from the rats and placed in a dish of ice-cold phosphate-buffered saline (PBS) for assays of α-amylase activity, and in Minimum Essential Medium (MEM) for ^{3}H-leucine incorporation studies. After removing the connective

tissue, nerves and large ducts in the central region, the glands were sliced with the Stadie–Riggs tissue slicer for incubation with the radioactive amino acid.

^{3}H-leucine incorporation studies

Tissue slices were collected in a Petri dish containing ice-cold MEM which had been gassed with 95% o_2 and 5% CO_2 and divided into groups for incubation. Each group was placed in a 25 ml Erlenmeyer flask containing 5 ml of MEM without leucine, to which 3 μCi/ml of L-[4,5,^{3}H(N)]-leucine (sp. act. 47–52 Ci/mmol; New England Nuclear, Boston, Mass.) was added and supplemented with ^{1}H-leucine to a final concentration of 0.1 mM. Flasks containing tissue slices and medium were continuously gassed and kept on ice for 10 min for equilibration. After this, flasks were transferred to a water bath maintained at 37°C and agitated at about 90 c/min while maintaining the 95% O_2–5% CO_2 atmosphere. At the end of the incubation, flasks were put on ice and tissues were removed from the medium by filtering through miracloth. Tissues were further rinsed with cold PBS and homogenized. A known amount of the homogenate was removed and used to determine the total radioactivity. The remaining homogenate was precipitated with cold perchloric acid (PCA) : (final concentration, 0.32M) and fractionated to obtain PCA-insoluble protein and extract DNA, as described below. The supernatant was used to determine the ^{3}H-leucine available in the PCA-soluble pool. The PCA-insoluble pellet was dissolved in NCS tissue solubilizer (Amersham, Chicago, Il.) following the extraction of DNA. Radioactivity in acid soluble fractions and in dissolved pellets was counted in a Packard Scintillation Spectrometer. Counting rates were corrected for quenching and background with an external standard. The total leucine incorporated into proteins was calculated from the total radioactivity and the specific activity of ^{3}H-leucine in the medium.

A parallel aliquot of the homogenate was precipitated with PCA (final concentration of 0.32M) and fractionated as described by Hinrichs et al. (1964). DNA was extracted with hot (70°C) 0.54M PCA from the resulting pellet and the amount of it was determined according to the diphenylamine method of Burton (1956), using calf thymus DNA (Sigma, St. Louis, Mo.) as the standard.

Assays of α-amylase activity

The glandular α-amylase was extracted by an extensive homogenization of the tissue in PBS (pH 6.9; 3.0 ml/ 0.1 g wet weight of the gland). A small amount of the homogenate was removed, centrifuged at about 25,000 g for 10 min, and the supernatant was used to assay for its α-amylase activity following appropriate dilutions. The amylase activity was assayed by using the Amylochrome method (Roche Diagnostics, Nutley, N.J.), which is a colorimetric procedure of measuring the amount of dye released from the Cibachrone Blue F_3 GA-amylose complex upon breaking of the 1–4 bonds of the amylose by the action of α-amylase.

Light microscopy

The glands were fixed by perfusing the animal through the heart with a mixture of 1% paraformaldehyde and 1.5% glutaraldehyde buffered with 0.1M sodium cacodylate to pH 7.4. The glands were removed, sliced into smaller pieces and further fixed in the same fixative for an additional hour. The tissues were post fixed in 1% solution of OsO_4 and prepared for microscopy as described previously (Kim et al., 1980). For light microscopy, 1 μm thick sections were cut and stained with 1% toluidine blue for viewing.

RESULTS

The rate of incorporation of ^{3}H-leucine into proteins, as well as the cellular level of α-amylase activity among the age groups, were compared based on the DNA content of the tissue. The wet weight of the tissue was not used as the index to avoid possible fluctuations in the values due to the increase in the glandular contents of fat and other nonsecretory tissues with aging.

The incorporation of ^{3}H-leucine into proteins

When the pancreatic or parotid gland slices were incubated in MEM supplemented with ^{3}H-leucine, label was incorporated into acid-insoluble protein at a constant rate for at least 3 h (unpublished observation). Therefore, the gland slices were routinely incubated for 30 min in this study when comparing the rate of label incorporation among the age groups.

The amount of leucine incorporated into the pancreatic protein, calculated from the total radioactivity in PCA-insoluble fractions, varies with age (Table 1). The amino acid

TABLE 1. THE INCORPORATION OF LEUCINE
INTO ACID-INSOLUBLE PROTEINS BY THE RAT
PANCREAS

Age (months)	nmole/mg DNA/h ± S.E.
2	88.97 ± 8.13[a, b, c]
12	121.67 ± 9.20[b, c, d]
18	95.83 ± 5.78[c, d]
24	56.03 ± 4.04[d]
30	44.23 ± 2.33

[a]Value differs significantly from the 12-month value ($p \leq 0.05$).
[b]Value differs significantly from the 18-month value ($p \leq 0.05$).
[c]Value differs significantly from the 24-month value ($p \leq 0.05$).
[d]Value differs significantly from the 30-month value ($p \leq 0.05$).

incorporation reaches the highest level at 12 months and decreases progressively with age. The level of incorporation is greatly reduced in 24- and 30-month-old rats and amounts to only 46 and 36% respectively, of the level observed in 12-month-old rats. Although it seems that the leucine incorporation at 18 months is higher than at 2 months, the difference between the two age groups is not significant. However, the level of incorporation of leucine at these two ages is significantly higher than that detected at 24 and 30 months.

On the other hand, the amount of leucine incorporated into the acid-soluble fractions of the pancreas does not vary significantly among the age groups. There is no significant difference in the amount of leucine incorporated into the acid-soluble fractions from the pancreas of 2-, 12- and 24-month-old rats (Table 2).

TABLE 2. THE INCORPORATION OF LEUCINE
INTO THE ACID SOLUBLE FRACTION OF THE
PANCREAS

Age (months)	nmole/mg DNA/h ± S.E.
2	0.303 ± 0.051
12	0.313 ± 0.040
24	0.418 ± 0.037

In the parotid gland (Fig. 1), the cellular level of leucine incorporation into protein is highest in 2-month-old rats and it declines progressively with age. The level of incorporation is reduced by 21, 29, 49 and 58% of the 2 month level in 12-, 18-, 24- and 30-month-old rats, respectively.

The cellular level of α-amylase activity

The α-amylase activity was assayed by using the Amylochrome method because this procedure proved to be more sensitive and consistent than Bernfeld's technique (Bernfeld 1955). When tested by using hog pancreas amylase (obtained from Sigma St. Louis, Mo.), 1050 dye units were comparable to 1 unit of α-amylase activity which produces 1 mg of maltose from starch in 3 min at 37°C.

The cellular level of α-amylase activity detected in the pancreas is lowest in young (2-month-old) rats, but reaches the highest level among all the age groups at 18 months.
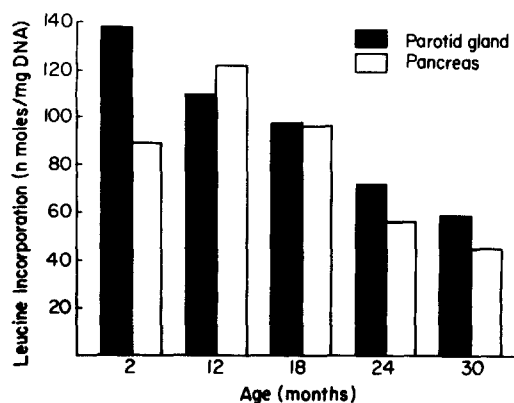
FIG. 1. Comparisons of the incorporation of ³H-leucine into PCA-insoluble proteins of the rat pancreas and parotid gland at different ages. The highest level of incorporation occurs at 2 months in parotid glands and at 12 months in the pancreas. After this, the rate of incorporation declines with age in both glands.

The cellular level of the enzyme activity is significantly reduced to about 50% of the 18-month level at 30 months (Table 3).

TABLE 3. AGE-RELATED DIFFERENCES IN α-AMYLASE ACTIVITY IN THE RAT PANCREAS

Age (months)	Dye units/mg tissue $\pm$ S.E. ($\times 10^3$)	Dye units/mg DNA $\pm$ S.E. ($\times 10^6$)
2	29.6 $\pm$ 2.3[a, b, c, d]	6.3 $\pm$ 0.5[a, b, c, d]
12	67.0 $\pm$ 4.2[c, d]	15.4 $\pm$ 0.9[b, c, d]
18	77.0 $\pm$ 3.9[c, d]	20.3 $\pm$ 1.0[c, d]
24	51.1 $\pm$ 2.6	14.7 $\pm$ 0.8[d]
30	46.8 $\pm$ 4.4	11.3 $\pm$ 0.1

[a]Value differs significantly from the 12-month value ($p \leq 0.05$).
[b]Value differs significantly from the 18-month value ($p \leq 0.05$).
[c]Value differs significantly from the 24-month value ($p \leq 0.05$).
[d]Value differs significantly from the 30-month value ($p \leq 0.05$).

The microscopic appearances of the pancreas

The differences in the cellular level of α-amylase activity detected in these age groups are well reflected in the number of secretory granules present in the acinar cells. Although the number of secretory granules per cell has not been counted, it is quite obvious that the secretory acinar cells of the pancreas of 18-month-old rats (Fig. 2b) contain many more granules than those cells of the gland from 2- (Fig. 2a) and 30-month-old rats (Fig. 2c). The cells of the pancreas from 2-month-old rats contain the smallest number of granules which are concentrated around the lumen. However, it appears that the secretory lumens of the pancreas in these young rats are more dilated than those in older rats, which may indicate a high level of secretory activity in the pancreas of young rats.

One noticeable difference detectable at 24 and 30 months is the presence of fat in the pancreases of these old rats. Fat globules are mostly located at the periphery of the lobules or in the connective tissue adjacent to the lobules. However, there is no detectable difference in the amount of fat present in the pancreases in these two age groups.
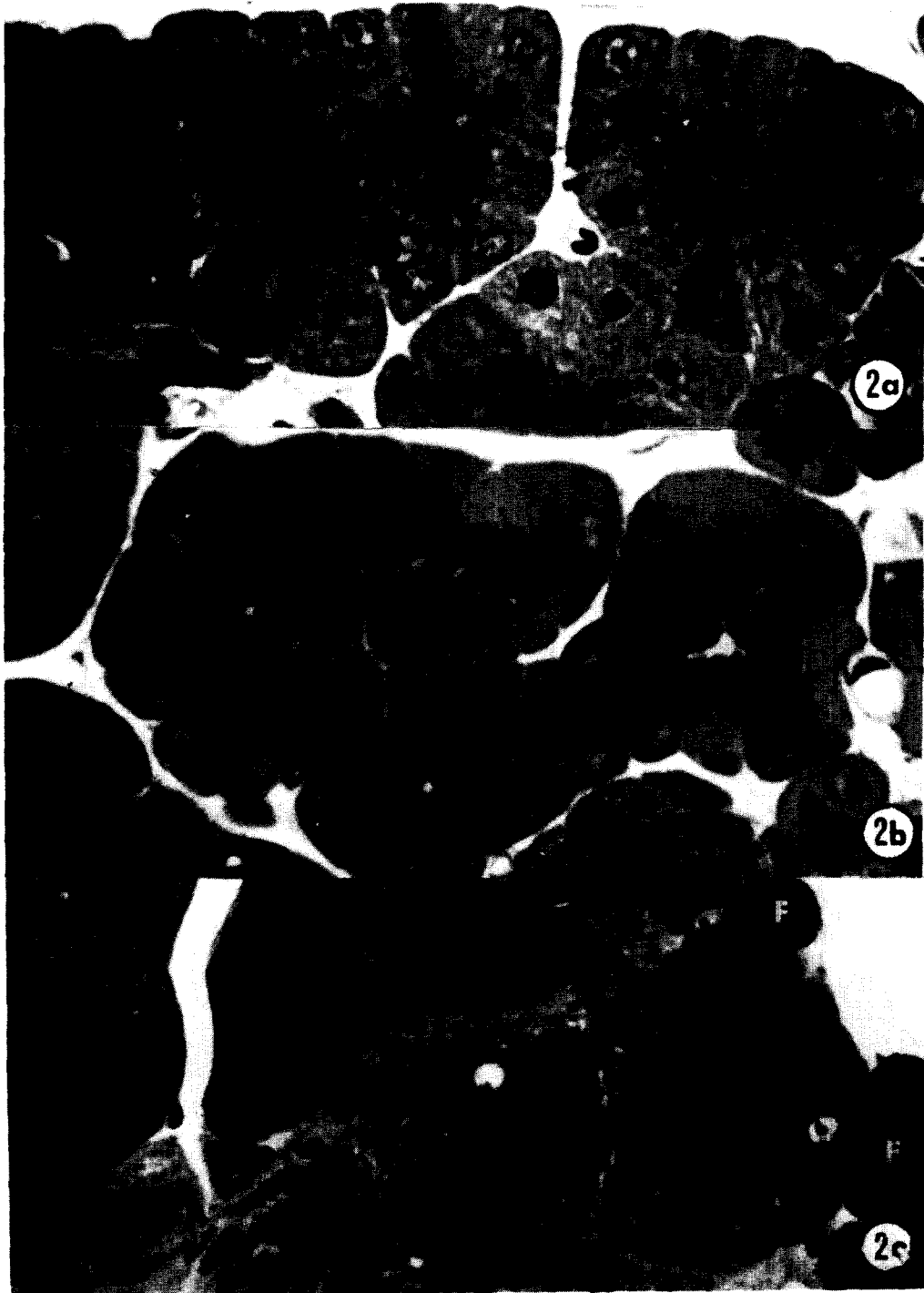
FIG. 2. Light micrographs ot the pancreas from 2- (a), 18- (b) and 30-month-old (c) rats. The largest number of secretory granules is present in the acinar cells at 18 months and this correlates with the highest level of α-amylase activity detected at this age. The number of secretory granules present in the pancreas of 2-month-old rats is smallest among the three age groups. This reduced number of granules may have resulted from a high level of secretory activity as indicated by the dilated lumens (L) of the acini. Fatty tissue present in the gland at 30 months is illustrated (F). × 900

DISCUSSION

The gradual reductions in the rate of leucine incorporation into the acid-insoluble protein shown by the pancreatic slices of the rats of increasingly older age indicate that protein synthesis declines with age. It appears that the cellular protein synthesis in the pancreas reaches the highest level at 12 months, and thereafter it declines progressively with age. The protein synthesis declines also in parotid glands of the old rats. However, the highest level of protein synthesis occurs at 2 months (Kim et al., 1980; Kim, 1980).

The decline in protein synthesis in the pancreas does not appear to be related to changes in the size of the free leucine pool, as is also the case in parotid glands (Kim et al., 1980; Kim, 1981). There is no significant change in the amino acid incorporation into acid-soluble fractions of the pancreas and parotid glands from various age groups. The free amino acid pool does not appear to change with age in other organs as well. For example, the level of total amino acids (Eichholz and Buetow, 1978) and the leucine pool (Salatka et al., 1971) in the livers of the rat and mouse do not change with age.

The decline in protein synthesis observed in the pancreas and parotid gland of the rat in this study is consistent with the findings of previous studies of other organs and tissues. Previous studies of in vitro amino acid incorporation in a cell free system have shown that the microsomes from older animals are less active in protein synthesis than those from younger animals (Breuer and Florini, 1965; Murthy, 1966; Hrachovec, 1969; Mainwaring, 1969; Srivastava, 1969; Buetow and Gandhi, 1973; Comolli, 1973; Britton and Sherman, 1975; Kurtz, 1975; Comolli et al., 1976). It is uncertain as to what factors are responsible for the reduction in the rate of amino acid incorporation by the microsomes of old animals.

The factors responsible for the age-related reduction in amino acid incorporation have been suggested to be the inhibitory substances in the cytosol (Murthy, 1966; Hrachovec, 1969; Buetow and Gandhi, 1973; Comolli, 1973; Comolli et al., 1976), or the changes in ribosomes (Mainwaring, 1969; Wallach and Gershon, 1974; Layman et al., 1976). It appears that the number of active ribosomes decreases with age (Kurtz, 1978) and, thus, reduces the synthetic capacity of the cells. Recently, it has been shown that the chain elongation factor (EF-1), prepared from the rat liver and brain of 30-month-old animals, has 30–40% less activity than the same factor prepared from 2-month-old animals (Moldave et al., 1979). The factor(s) responsible for the reduction in protein synthesis in the pancreas with aging remains to be studied.

The differences in the cellular level of α-amylase activity detected in the pancreas among the age groups correlate well with the differences in the number of secretory granules present in the acinar cells of these groups. Thus, it is very likely that the detected levels of α-amylase activity reflect the cellular contents of secretory proteins. Based on the amylase activity data, the highest level of secretory proteins is present in the pancreas at 18 months and the lowest level at 2 months. The secretory protein content of the pancreas at 24 and 30 months is higher than that at 2 months. Therefore, the changes in the cellular contents of secretory proteins do not parallel the changes in protein synthesis as detected by the differences in the leucine incorporation into the pancreatic protein.

However, the cellular level of secretory proteins (enzymes) probably does not depend on the level of protein synthesis alone. It is likely that the cellular level of secretory proteins reflects the net effect of the levels of protein synthesis and secretory activity of the cells. The morphological appearance of the pancreas at 2 months supports this

point. The secretory lumens of the pancreatic acini at this age are widely dilated, indicating a high level of secretory activity of the acinar cells. The dilated lumen results from the fusion of the secretory granule membranes with the apical cell membrane during the secretion of the granule contents (Amsterdam *et al.,* 1969). Then, the small number of secretory granules present in the acinar cells of 2-month-old rats are likely to be due to the high level of secretory activity of the gland at this age.

The discrepancy between the cellular level of α-amylase activity and the rate of protein synthesis also exists in the parotid gland of the same age groups (Kim, 1981). Although the highest level of protein synthesis is detected in parotid glands of 2-month-old rats, the highest level of amylase activity is detected in 12-month-old rats. Furthermore, the rate of protein synthesis is reduced by over 50%, while the content of α-amylase is reduced by only about 25% between the age of 2 and 30 months. Therefore, it appears that there is no parallel relationship between the cellular contents of secretory proteins and the rate of protein synthesis in the rat pancreas and parotid glands.

Nevertheless, it is clear that the rate of protein synthesis declines with age in both the pancreas and parotid gland, although the level of protein synthesis in parotid glands is highest at 2 months and declines progressively with age. The difference in the pattern of changes in protein synthesis observed in these two digestive glands indicates that the age-related changes in protein synthesis reflect the functional changes in each gland rather than an overall decline in the synthetic activity of the secretory cells. In fact, we have shown that the age-related decline in protein synthesis detected in rat parotid glands occurs independent of the changes in the cellular metabolic activity or the synthesis of lipids (Kim *et al.,* 1979, 1980).

SUMMARY

This study was undertaken to determine whether changes occur in protein synthesis with age in the pancreas of the rat and to compare the pattern of changes with that observed in parotid salivary glands. The rate of incorporation of ^{3}H-leucine into acid-insoluble proteins declines with age in both glands. In the pancreas, the rate of incorporation reaches the highest level at 12 months and declines by 21, 54 and 64% of this level at 18, 24 and 30 months, respectively. In parotid glands, the highest level of the amino acid incorporation occurs at 2 months and the level declines by 21, 29, 49 and 58% of the 2-month level at 12, 18, 24 and 30 months, respectively. There is no age-related difference in the rate of incorporation of ^{3}H-leucine into acid-soluble fractions of the two glands. The cellular level of α-amylase is also reduced in the pancreas and parotid gland of old (24- and 30-month-old) rats. The differences in the cellular level of α-amylase activity among the age groups correlate with the differences in the number of secretory granules present in the acinar cells of the pancreas, indicating that the level of amylase reflects the cellular content of secretory proteins. There is no detectable morphological change that parallels the over 50% reduction in protein synthesis in these glands of young and old rats.

REFERENCES

AMSTERDAM, A., OHAD, I. and SCHRAMM, M. (1969) *J. Cell Biol.* **41**, 753.
BARTOS, V. and GROH, J. (1969) *Gerontol. Clin.* **11**, 56.

BERNFELD, P. (1955) *Methods in Enzymology* (Edited by S. D. COLOWICK and N. O. KAPLAN). p. 149. Academic Press, New York.

BERTRAM, U. (1967) *Acta odontol. Scand.* 25 Suppl. **49**, 1.

BREUER, C. B., DAVIES, M. C. and FLORINI, J. R. (1964) *Biochemistry* 3, 1713.

BREUER, C. B. and FLORINI, J. R. (1965) *Biochemistry* 4, 1544.

BRITTON, G. W. and SHERMAN, F. G. (1975) *Exp. Gerontol.* **10**, 67.

BUETOW, D. E. and GANDHI, P. S. (1973) *Exp. Gerontol.* **8**, 243.

BURTON, D. (1956) *Biochem. J.* **62**, 315.

CHILLA, R., NIEMANN, H., ARGLEBE, C. and DOMAGK, G. F. (1974) ORL—*J. Oro-Rhino-Laryngol. its Borderl.* **36**, 372.

COMOLLI, R. (1973) *Exp. Gerontol.* **8**, 307.

COMOLLI, R., DELPIANO, C. and SHUBERT, A. C. (1976) *Exp. Gerontol.* **11**, 5.

EICHHOLZ, R. L. and BUETOW, D. E. (1978) *Gerontologia* **24**, 32.

HINRICHS, H. R., PETERSON, R. O. and BASERGA, R. (1964) *Arch. Pathol.* **78**, 245.

HRACHOVEC, J. P. (1969) *Gerontologia* **15**, 52.

JOHNSON, T. C. (1968) *J. Neurochem.* **15**, 1189.

JOHNSON, T. C. and BELYTSCHKO, G. (1969) *Proc. Natn. Acad. Sci., U.S.A.* **62**, 844.

KIM, S. K., WEINHOLD, P. A., WAGNER, D. J. and HAN, S. S. (1979) *J. Cell Biol.* **83**, 439a.

KIM, S. K., WEINHOLD, P. A., HAN, S. S. and WAGNER, D. J. (1980) *Exp. Gerontol.* **15**, 77.

KIM, S. K. (1981) *J. Dent. Res.* In press.

KLETHI, J. (1976) *Invest. Ophthalmol.* **15**, 430.

KURTZ, D. I. (1975) *Biochim. biophys. Acta* **407**, 479.

KURTZ, D. I. (1978) *Exp. Gerontol.* **13**, 397.

LAYMAN, D. K., RICCA, G. A. and RICHARDSON, A. (1976) *Archs Biochem. Biophys.* **173**, 246.

LIU, D. S. H., EKSTROM, R., SPICER, J. W. and RICHARDSON, A. (1978) *Exp. Gerontol.* **13**, 197.

MAINWARING, W. I. P. (1968) *Biochem. J.* **110**, 79.

MAINWARING, W. I. P. (1969) *Biochem. J.* **113**, 869.

MEYER, J., GOLDEN, J. S., STEINER, N. and NECHELES, H. (1937) *Am. J. Physiol.* **119**, 600.

MEYER, J. and NECHELES, H. (1940) *J. Am. med. Assoc.* **115**, 2050.

MOLDAVE, K., HARRIS, J., SABO, W. and SADNIK, I. (1979) *Fedn. Proc. Fedn. Am. Socs. exp. Biol.* **38**, 1979.

MURTHY, M. R. V. (1966) *Biochim. biophys. Acta* **119**, 599.

NECHELES, H., PLOTKE, F. and MEYER, J. (1942) *Am. J. Dig. Dis.* **9**, 157.

ROSENBERG, I. R., FRIEDLAND, N., JANOWITZ, H. D. and DREILING, D. A. (1966) *Gastroenterology* **50**, 191.

SALATAKA, K., KRESGE, D., HARRIS, L., JR., EDELSTEIN, D. and OVE, P. (1971) *Exp. Gerontol.* **6**, 25.

SRIVASTAVA, U. (1969) *Archs Biochem. Biophys.* **130**, 129.

WALLACH, Z. and GERSHON, D. (1974) *Mech. Age. Dev.* **3**, 225.