Age-related changes in albumin elimination in female WAG/Rij rats

G. J. M. Jean HORBACH,* Sing-Hiem YAP† and Cornelis F. A. VAN BEZOOIJEN*

*Institute for Experimental Gerontology TNO, P.O. Box 5815, 2280 HV Rijswijk, The Netherlands, and

†Division of Gastrointestinal and Liver Diseases, Department of Medicine, University of Nijmegen, Nijmegen, The Netherlands

(Received 14 February 1983/Accepted 1 August 1983)

Albumin elimination rates were determined in 3-, 12-, 24- and 36-month-old female WAG/Rij rats. No change in elimination half-life was found with age. However, as there was an increase in the whole-body albumin pool, a concomitant increase in albumin clearance was observed at between 12 and 36 months of age. It was concluded that the increase in clearance between 12 and 24 months of age was only due to a change in the animal's physiology, whereas between 24 and 36 months of age it was also due to changes in the albumin molecule. The age-related changes in albumin clearance were thought not to be caused by changes in the albumin excretion via the urine or via the gastrointestinal tract.

Proteins play essential roles in the function and viability of cells in all living organisms. Therefore changes in protein synthesis and/or elimination‡ could be among the most important mechanisms involved in aging. Although the age-related changes in protein synthesis have been extensively studied over the last decade, only a little information is available on the age-related changes in protein elimination (Richardson, 1981; Richardson & Birchenall-Sparks, 1983).

Since the liver has a high protein-synthesizing activity, this organ has been chosen by many investigators for the study of changes in the capacity for protein synthesis with age (for a review, see van Bezooijen, 1978). One of the few specific liver proteins of which the age-related changes in its synthesis has been studied is albumin. Studies *in vitro* with liver microsomes isolated from male Fischer 344 rats showed an increase in albumin synthesis between 1.5- and 24-month-old animals (Chen *et al.*, 1973). van Bezooijen and co-workers showed that the capacity of hepatocytes isolated from female WAG/Rij rats to synthesize albumin was less at 24 months than at 3 and 12 months of age, whereas it was higher at 30 and 36 months of age as compared with the 12- and 24-month values (van Bezooijen *et al.*, 1976; van Bezooijen & Knook, 1977). Studies *in vivo* on age-related changes in albumin synthesis were performed by

Beauchene *et al.* (1970), Salatka *et al.* (1971) and Ove *et al.* (1972). They all found an increase in albumin synthesis with age among the age groups investigated: 11- and 28-month-old male and female Wistar rats (Beauchene *et al.*, 1970), 1.5- and 20-month-old female Fischer rats (Salatka *et al.*, 1971) and 1- and 20-month-old female Fischer rats (Ove *et al.*, 1972). With regard to age-related changes in albumin elimination, only a few reports have been published (Salatka *et al.*, 1971; Ove *et al.*, 1972; Quinn *et al.*, 1973). These investigators, however, expressed the elimination rate of albumin as a relative index, the elimination half-life, $t_{1/2}$,_{el}. Only Quinn *et al.* (1973) found a change in this index with age in mice, where the elimination half-life was sharply increased very late in life. Because they only looked at the plasma compartment and not the whole-body albumin pool, they were not able to determine the absolute albumin elimination rate. This study will focus on the age-related changes in the absolute albumin elimination rate and on the contribution of urinary excretion to it.

Materials and methods

Animals and materials

Female WAG/Rij rats were maintained under 'clean conventional' conditions as described in detail by Hollander (1976). The average weights of the 3-, 12-, 24- and 36-month-old animals were 151 ± 13 , 182 ± 16 , 208 ± 16 and 186 ± 20 g respectively (mean $\pm$ S.D.).

‡ In the present paper we define elimination as the sum of all the degradation and excretion processes.

DEAE Affi-Gel Blue was obtained from Bio-Rad Laboratories, Richmond, CA, U.S.A. Goat anti-(rat total serum proteins) antiserum used for immunoelectrophoresis (GARa/ielfo) and rabbit anti-(rat serum albumin) antiserum (RARa/alb) were obtained from Nordic Immunological Laboratories, Tilburg, The Netherlands. $^{51}\text{CrCl}_3$ and ^{125}I were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Other chemicals of a high grade were obtained from standard chemical suppliers. Minicon B-15 macrosolute concentrators were obtained from Amicon Ltd., Woking, Surrey, U.K.

Albumin isolation, purification and labelling with ^{125}I

The isolation and purification of albumin was achieved by chromatography of fresh rat serum on DEAE Affi-Gel Blue. DEAE Affi-Gel Blue is an affinity matrix made by coupling Cibracon Blue F3GA to specially prepared DEAE-Bio-Gel A. A column with a total bed volume of 7 ml/ml of serum was prepared. The column was prewashed with two bed volumes of 0.02 M- K_2HPO_4 , pH 8.0 (Buffer 1). The serum sample was dialysed for 24 h against Buffer 1. The serum sample was applied to the column and the column was washed with 3–4 bed volumes of Buffer 1, 2 bed volumes of 0.1 M- K_2HPO_4 , pH 8.0, 2 bed volumes of 0.2 M- K_2HPO_4 , pH 8.0, 1 bed volume of 0.4 M- K_2HPO_4 , pH 8.0, and 2 bed volumes of Buffer 1. The column was eluted with 2 bed volumes of 1 M-KSCN. The albumin and the KSCN were separated by chromatography on a Sephadex G-50 (medium grade) column (1.6 cm $\times$ 18.0 cm) equilibrated with phosphate-buffered saline (24 mM - KH_2PO_4 /63 mM - K_2HPO_4 /154 mM - NaCl, pH 7.4). The albumin-containing fractions were pooled and concentrated on Minicon B-15 concentrators to a final concentration of ± 10 mg/ml.

The yield was approx. 50%. Immunoelectrophoresis established the purity of the isolated albumin (purity, $\pm 99\%$) (Fig. 1). Rat serum albumin was iodinated by the method of McFarlane (1956) as modified by Helmkamp *et al.* (1967) to a final ratio of 1 mol of ^{125}I /mol of albumin.

Plasma radioactivity curves

Iodinated albumin (5 μCi) was injected intravenously into the jugular vein of rats of the different age groups. Trichloroacetic acid-precipitable radioactivity, with a bovine serum albumin/KI solution as a carrier, was determined in 20 μl of plasma at several time points during 5 days. Blood was sampled from the tail. Radioactivity was directly counted in a γ -radiation counter. From the plasma radioactivity curves the following characteristics were calculated: (a) elimination half-life; $t_{1/2\text{el}}$; (b) apparent volume of distribution, V_d ; (c) clearance, Cl.

The elimination half-life is defined as the time required for the body to eliminate one-half of the ^{125}I -albumin which it contains; it may be calculated by:

$$t_{1/2\text{el}} = \frac{\ln 2}{\beta} \text{ (h)}$$

where β is the terminal slope in the plasma activity curve. The elimination half-life is an index for the relative elimination rate.

The apparent volume of distribution is defined as the volume of body water that would be required to contain the amount of ^{125}I -albumin in the body if it were uniformly present in the same concentration in which it is in the plasma. Since all compartments that contain the ^{125}I -albumin may not have equal concentrations, this volume calculated by using the ^{125}I -albumin concentration in only one compartment (which is the plasma) can only be an apparent volume. V_d may be calculated by:

$$V_d = \frac{D}{(\text{AUC}) (\beta)} \text{ (ml)}$$

where D is the amount injected and AUC is the area under the plasma activity curve from zero time to infinity. V_d is a proportionality factor which, when multiplied by the ^{125}I -albumin concentration in the plasma, yields the amount of ^{125}I -albumin present in the body. The use of V_d as a proportionality factor is valid only after the establishment of an equilibrium between the different compartments.

The clearance can be defined as the amount of plasma which is totally cleared of ^{125}I -albumin per unit of time. The clearance can be calculated as:

$$\text{Cl} = \frac{D}{\text{AUC}} \text{ (ml/h)}$$

The clearance is used as an index for the absolute albumin elimination rate. These characteristics have been defined by Gibaldi & Perrier (1975).

Albumin concentration in urine and plasma

The albumin concentration in urine and plasma was determined by radial immunodiffusion as described by Mancini *et al.* (1965).

The whole-body albumin pool and the absolute and fractional rate of albumin elimination

With the use of the plasma albumin concentration, the data for apparent volume of distribution and clearance can be extrapolated in terms of the whole-body albumin pool and the absolute and fractional rates of albumin elimination. The whole-body albumin pool can be calculated by:

$$V_d \times \text{plasma albumin concentration (mg)}$$

Fig. 1. *Immunoelectrophoresis of rat serum albumin (RSA) purified from female WAG/Rij rats of different ages by DEAE Affi-gel Blue chromatography*

Well A contained serum from 3-month-old female WAG/Rij rats. Wells B–E contained RSA isolated from 3-, 12-, 24- and 36-month-old rats respectively. Well F contained commercially available RSA (Nordic) (purity 98.5%). Goat anti-(rat total serum proteins) antiserum was used.

The absolute rate of albumin elimination can be calculated by:

$$Cl \times \text{plasma albumin concentration} \times 24 \text{ (mg/24 h)}$$

The fractional rate of albumin elimination is the absolute rate of albumin elimination expressed as a percentage of the whole-body albumin pool per day. It can be calculated by:

$$\frac{\text{Absolute rate of albumin elimination} \times 100}{\text{Whole-body albumin pool}} \text{ (%/24 h)}$$

Quantitative estimation of gastrointestinal protein loss

Protein loss through the intestinal wall was determined by using the $^{51}\text{CrCl}_3$ method described by van Tongeren & Reichert (1966). $^{51}\text{CrCl}_3$ (5 μCi) was injected intravenously into the jugular vein of

rats of the four age groups. Radioactivity was determined in 20 μl of plasma at several time points during 30 h after injection. At 24 h after injection, 25 mg of Carmine were administered orally to the rats as a faeces marker. Radioactivity in the faeces was determined until the appearance of the marker there.

Results

Plasma radioactivity curves

Plasma clearance studies were made in each rat of the different age groups with two different albumins. Firstly, each rat was injected intravenously with albumin isolated from 3-month-old rats. In this approach, age-related changes in the elimination of the albumin were due only to physiological changes in the animal.

Secondly, the same rats were injected intravenously with albumin isolated from rats of the same age as the recipient. This approach revealed age-related changes in the elimination of albumin due to a combination of physiological changes in the animal and changes in the albumin molecule.

Plasma radioactivity of albumin isolated from 3-month-old rats was plotted semilogarithmically against the time after injection. Fig. 2 shows the mean plasma radioactivity curves for the four age

Fig. 2. Mean plasma radioactivity curves obtained after injection of albumin isolated from 3-month-old rats into rats of different age groups

^{125}I -albumin ($5\mu\text{Ci}$) was injected intravenously. Trichloroacetic acid-precipitable radioactivity was determined in $20\mu\text{l}$ of plasma at several time points during 5 days. Radioactivity at zero time was extrapolated from determinations after 2, 3 and 5 min. The values represent the means $\pm$ S.D. for a minimum of six experiments.

groups. From the individual plasma radioactivity curves, the elimination half-life, the apparent volume of distribution and the clearance were calculated. Age-related changes in these three characteristics are shown in Table 1. Although there are no major changes with age in the elimination half-lives for albumin, the values for clearance and apparent volume of distribution are higher at 24 months of age than at 3 and 12 months of age. At 36 months of age, only the clearance is significantly higher than at 3 months of age.

Plasma clearance studies of albumin isolated from rats of the same age as the recipient were made in the same rats as used for the elimination studies mentioned above. Mean plasma radioactivity curves for the four age groups are shown in Fig. 3. Age-related changes in the characteristics calculated from the individual plasma radioactivity curves are shown in Table 1. Again, no significant changes with age in elimination half-lives of these albumins are observed. The values for clearance and apparent volume of distribution are higher at 24 and 36 months of age than those at 3 and 12 months of age. In contrast with the results obtained from clearance studies with albumin isolated from 3-month-old rats, higher values for apparent volume of distribution and clearance at 36 months of age as compared with 24-month-old animals are observed.

The influence of the age of the rats from which the albumin was isolated on $t_{1/2}$, V_d and Cl is shown in Table 1. Albumin isolated from rats of the same age as the recipient and from 3-month-old rats was administered to 12-, 24- and 36-month-old rats;

Table 1. Age-related changes in elimination half-life ($t_{1/2}$), apparent volume of distribution (V_d) and clearance (Cl) of radioiodinated albumin in female WAG/Rij rats

Albumin isolated from rats of the same age as the recipients and from 3-month-old rats was administered to 12-, 24- and 36-month-old rats; albumin isolated from 3- and 36-month-old rats was administered to 3-month-old ones. From the individual plasma radioactivity curves obtained in this way the metabolic characteristics were determined by calculation. Values are expressed as means $\pm$ S.D. Key to superscripts: ^avalue differs significantly ($P < 0.05$) from 3-month value; ^bvalue differs significantly ($P < 0.05$) from 12-month value; ^cvalue differs significantly ($P < 0.05$) from 24-month value; ^dvalue differs significantly ($P < 0.05$) from that obtained when albumin isolated from 3-month-old rats was used.

Albumin-donor-rat age	Parameter	Age of the recipient (months)			
		3	12	24	36
3 months	n	6	6	7	7
	$t_{1/2}$, el (h)	44.3 ± 3.4	43.6 ± 7.9	41.5 ± 4.0	39.0 ± 5.3
	Cl (ml/h)	0.226 ± 0.028	0.263 ± 0.047	$0.356 \pm 0.063^{\text{ab}}$	$0.308 \pm 0.071^{\text{a}}$
	V_d (ml)	14.4 ± 1.6	15.9 ± 2.0	$20.6 \pm 4.4^{\text{ab}}$	17.3 ± 3.4
Same age as recipient	n	6	6	7	11
	$t_{1/2}$, el (h)	44.3 ± 3.9	46.2 ± 6.1	39.6 ± 4.7	40.3 ± 4.6
	Cl (ml/h)	0.226 ± 0.028	0.244 ± 0.038	$0.359 \pm 0.067^{\text{ab}}$	$0.420 \pm 0.056^{\text{abcd}}$
	V_d (ml)	14.4 ± 1.6	16.7 ± 2.9	$20.2 \pm 2.7^{\text{ab}}$	$24.0 \pm 4.1^{\text{abcd}}$
36 months	n	6			
	$t_{1/2}$, el (h)	42.9 ± 4.8			
	Cl (ml/h)	0.277 ± 0.050			
	V_d (ml)	16.4 ± 3.9

Fig. 3. Mean plasma radioactivity curves obtained after injection of albumin isolated from rats of the same age as the recipient into rats of different age groups ¹²⁵I-albumin was used. Experimental details are given in Fig. 2. The values represent the means ± S.D. for a minimum of six experiments.

albumin isolated from 3- and 36-month-old rats was administered to 3-month-old ones. Significantly higher values for apparent volume of distribution and clearance are found only in 36-month-old rats when comparing albumin isolated from rats of the same age as the recipient and that isolated from 3-month-old animals. In the rats of the other three age groups, no changes were found in these characteristics when albumin isolated from rats of a different age group was administered.

Albumin concentration in plasma

Plasma albumin concentrations were determined in the same rats as used for constructing the plasma activity curves. The results are given in Table 2. No significant changes in the albumin concentration in plasma with age can be seen.

The whole-body albumin pool and the absolute and fractional rate of albumin elimination

From the values obtained for clearance, apparent volume of distribution and the plasma albumin concentration values for the whole-body albumin pool, the absolute and fractional rate of albumin elimination could be calculated. Since the results with regard to clearance and apparent volume of distribution showed an influence of the age of the albumin donor rats on these characteristics in 36-month-old rats (see Table 1), the calculations were carried out only on data obtained with age-matched albumin.

The results are shown in Table 2. No significant changes in the fractional rate of albumin elimination with age can be seen. However, the values for the whole-body albumin pool and for the absolute rate of albumin elimination were higher at 24 months of age than at 3 and 12 months of age, and at 36 months of age than at 3, 12 and 24 months.

Urinary excretion of albumin

Animals were kept in metabolic cages for 24 h and supplied with water and food *ad libitum*. The urine was collected and the amount of albumin excreted was determined. The results are presented in Table 2. These data on albumin excretion in the urine show an enormous increase with age. However, comparing the urinary albumin excretion with the total albumin elimination, it can be seen that the urinary excretion contributes only 0.1–2.4% to the total elimination of albumin in these rats (Table 2).

Gastrointestinal protein loss

Protein loss through the intestinal wall was determined with the ⁵¹CrCl₃ method. The results are given in Table 3. No significant changes in gastrointestinal protein loss with age can be seen.

Table 2. Influence of age on plasma albumin concentration, the whole-body albumin pool, the absolute and fractional rate of albumin elimination and the urinary albumin excretion in female WAG/Rij rats

Plasma and urinary albumin concentrations were determined in rats that were used in the elimination experiments. Albumin concentrations in plasma and urine were determined by radial immunodiffusion. The whole-body albumin pool and the absolute and fractional rates of albumin elimination were calculated from the values for apparent volume of distribution and for clearance obtained after injection of age-matched albumin. Values are expressed as means ± S.D. Numbers of animals are given in parentheses. Key to superscripts: ^avalue differs significantly (*P* < 0.05) from 3-month value; ^bvalue differs significantly (*P* < 0.05) from 12-month value; ^cvalue differs significantly (*P* < 0.05) from 24-month value.

Parameter	Age (months)	3	12	24	36
Plasma albumin concentration (mg/ml)		36.3 ± 3.4 (6)	39.4 ± 3.8 (6)	40.6 ± 3.8 (7)	38.2 ± 2.2 (11)
Whole-body albumin pool (mg)		522 ± 58 (6)	660 ± 145 (6)	821 ± 111 ^{ab} (7)	952 ± 109 ^{abc} (11)
Absolute rate of albumin elimination (mg/24 h)		197 ± 12 (6)	255 ± 53 (6)	353 ± 74 ^{ab} (7)	430 ± 70 ^{abc} (11)
Fractional rate of albumin elimination (% of whole albumin pool/24 h)		37.7 ± 3.8 (6)	38.6 ± 2.4 (6)	42.6 ± 5.5 (7)	43.0 ± 5.6 (11)
Urinary albumin excretion (mg/24 h)		0.22 ± 0.08 (6)	0.20 ± 0.11 (6)	2.4 ± 1.4 ^{ab} (7)	16 ± 16 ^{abc} (6)
Urinary excretion as percentage of absolute elimination rate		0.12 ± 0.06 (6)	0.09 ± 0.07 (6)	0.7 ± 0.5 ^{ab} (7)	2.4 ± 1.9 ^{abc} (6)

Table 3. *Influence of age on the gastrointestinal clearance (Cl gastro) of plasma proteins*

$^{51}\text{CrCl}_3$ ($5\mu\text{Ci}$) was injected intravenously into rats of the different age groups. Radioactivity was determined in $20\mu\text{l}$ of plasma at several time points during 30 h. At time t (± 24 h after injection), 25 mg of Carmine were administered orally to the rats as a faeces marker. Radioactivity in the faeces was determined until the appearance of the marker there. Gastrointestinal clearance was calculated by the quotient of the radioactivity in the faeces and the area under the plasma radioactivity curve from zero time to time t . Values are expressed as means $\pm$ s.d.

Age (months)	No. of animals	Cl gastro (ml/h)
3	7	0.161 ± 0.062
12	7	0.184 ± 0.084
24	6	0.130 ± 0.035
36	5	0.113 ± 0.021

Discussion

Several investigators have shown that the capacity of the rat liver to synthesize albumin is dependent on the age of the rat (Beauchene *et al.*, 1970; Salatka *et al.*, 1971; Ove *et al.*, 1972; Chen *et al.*, 1973; van Bezooijen *et al.*, 1976; van Bezooijen & Knook, 1977). By using different experimental procedures, rat strains and sexes, they all found elevated values for albumin synthesis in old rats. One of the possible explanations for the increase in albumin synthesis was that the liver had to compensate for an increase in albumin excretion via the urine due to kidney insufficiency, as suggested by Beauchene *et al.* (1970). Indeed, in a variety of rat strains, an increase in urinary excretion of albumin with age was found (Beauchene *et al.*, 1970; Obenrader *et al.*, 1974; Alt *et al.*, 1980). However, very little attention was paid to age-related changes in total albumin elimination. No changes in the elimination half-life for albumin with age were found in rats (Salatka *et al.*, 1971; Ove *et al.*, 1972), but no determination of the absolute elimination rate of albumin was made.

The work reported here showed age-related changes in the absolute albumin elimination rate in female WAG/Rij rats and, by determination of the urinary albumin excretion in the same rats, the contribution of the changes in this excretion to the changes in the total elimination. We have chosen an experimental set-up in which we were able to determine whether changes in albumin elimination were due to changes in the physiology of the animal and/or to changes in the albumin molecule.

After injection of albumin isolated from rats of the same age as the recipient, the clearance was found to change with age. The value for clearance was higher at 36 months of age than at 24 months of age. At 24

months of age a higher value for clearance as compared with 12-month-old rats was observed (Table 1). However, no change in elimination half-life for albumin with age was seen. This observation is in agreement with the results on elimination half-lives as published by Salatka *et al.* (1971) and by Ove *et al.* (1972). Concomitant with the increase in clearance with age, an increase in apparent volume of distribution was seen. Since the plasma albumin concentration was constant throughout the lifespan of female WAG/Rij rats, the increase in apparent volume of distribution reflected an increase in the whole-body albumin pool (Table 2).

After injection of albumin isolated from 3-month-old rats into these animals, no increase in clearance was found between 24- and 36-month-old rats. The increase in albumin clearance between 12- and 24-month-old rats, however, was still present (Table 1). Furthermore, rats of 12 and 24 months of age clear albumin isolated from rats of their own age in the same way as that isolated from 3-month-old ones (Table 1). These observations indicated that the increase in albumin elimination between 12- and 24-month-old rats was not caused by a change in the albumin molecule but by a change in the physiology of the animal.

The higher value for the absolute elimination rate of albumin in 36-month-old rats as compared with that of 24-month-old ones (Table 2) might be due to changes in the albumin molecule in this time interval. However, since 3-month-old rats did not eliminate the albumin isolated from 36-month-old ones in a way different from the elimination of their own albumin, the albumin of 36-month-old rats could not be the sole cause of the change in absolute albumin elimination rate at between 24 and 36 months of age (Table 1).

Table 2 shows the enormous increase in urinary albumin excretion at between 12 and 24, and 24 and 36, months of age. The age-related changes in absolute albumin elimination rate could not be attributed to this increase in urinary albumin excretion, since it was calculated that the high excretion rate at 36 months of age contributes only 2.4% to the total elimination of albumin in the old rats. Therefore, since the old rats were able to eliminate up to 98% of the eliminated albumin via pathways other than the urinary excretion, it could be concluded that the increase in albumin excretion in the urine was not the trigger for the increase in albumin synthesis with age in this rat strain, as suggested by Beauchene *et al.* (1970) for male and female Wistar rats.

Another excretion process that could influence the albumin elimination with age is protein loss through the gastrointestinal wall. Table 3 shows the influence of age on the gastrointestinal protein loss. It can be seen that the gastrointestinal clearance remained

constant with age. However, in this experiment no estimation of the albumin clearance through the intestinal wall could be made. After injection of $^{51}\text{CrCl}_3$ at least albumin and transferrin are labelled, with a selectivity for transferrin. So one must bear in mind that changes in ^{51}Cr clearance may be extrapolated to changes in albumin clearance only when no distinction is made between different plasma proteins by the intestinal wall during leakage (van Tongeren & Reichert, 1966).

The cause for the increase in the absolute albumin elimination rate with age is presently unknown. This should be the aim of future research.

These investigations were supported in part by the Foundation for Medical Research (FUNGO), which is subsidized by the Netherlands Organization for Advancement of Pure Research (ZWO). We thank Ms. Lutien Vermeer-Greven and Miss Marion Roggenkamp for the preparation of the manuscript. Dr. A. C. Ford is thanked for editing the English text before the manuscript was submitted.

References

- Alt, J. M., Hackbarth, H., Deerberg, F. & Stolte, H. (1980) *Lab. Anim.* **14**, 95–101
- Beauchene, R. E., Roeder, L. M. & Barrows, C. H. (1970) *J. Gerontol.* **25**, 359–363
- Chen, J. C., Ove, P. & Lansing, A. I. (1973) *Biochim. Biophys. Acta* **312**, 598–607
- Gibaldi, M. & Perrier, D. (1975) *Pharmacokinetics*, Marcel Dekker, New York
- Helmkamp, R. W., Contreras, H. A. & Bale, W. F. (1967) *Int. J. Appl. Radiat. Isot.* **18**, 737–746
- Hollander, C. F. (1976) *Lab. Anim. Sci.* **26**, 320–328
- Mancini, G., Carbonara, A. O. & Heremans, J. F. (1965) *Immunochemistry* **2**, 235–254
- McFarlane, A. S. (1956) *Biochem. J.* **62**, 135–143
- Obenrader, M., Chen, J., Ove, P. & Lansing, A. I. (1974) *Exp. Gerontol.* **9**, 173–180
- Ove, P., Obenrader, M. & Lansing, A. I. (1972) *Biochim. Biophys. Acta* **277**, 211–221
- Quinn, R. P., Price, G. B., Ellis, J. M. & Makinodan, T. (1973) *J. Gerontol.* **28**, 257–264
- Richardson, A. (1981) in *Handbook of Biochemistry in Aging* (Florini, J. R., ed.), pp. 79–101, CRC Press, Boca Raton, FL
- Richardson, A., & Birchenall-Sparks, M. C. (1983) in *Review of Biological Research in Aging*, vol. 1 (Rothstein, M., ed.), pp. 255–274, Alan R. Liss, New York
- Salatka, K., Kresge, D., Harris, L., Jr., Edelstein, D. & Ove, P. (1971) *Exp. Gerontol.* **6**, 25–36
- van Bezooijen, C. F. A. (1978) Ph.D. Thesis, University of Utrecht
- van Bezooijen, C. F. A. & Knook, D. L. (1977) in *Liver and Aging* (Platt, D., ed.), pp. 227–241, Schattauer Verlag, Stuttgart and New York
- van Bezooijen, C. F. A., Grell, T. & Knook, D. L. (1976) *Biochem. Biophys. Res. Commun.* **71**, 513–519
- van Tongeren, J. H. M. & Reichert, W. J. (1966) *Clin. Chim. Acta* **14**, 42–48