J. Anat. (1972), **113** 1, pp. 17–34
With 19 *figures*
Printed in Great Britain

The growth and development of the rat aorta

II. Changes in nucleic acid and scleroprotein content

T. LOOKER AND C. L. BERRY

Department of Pathology, Guy's Hospital Medical School, London, SE1 9RT

(*Accepted* 6 *July* 1972)

INTRODUCTION

We have previously described the morphological changes occurring in the rat aorta during growth and development (Berry, Looker & Germain, 1972*a*). The purpose of the present report is to describe changes in the nucleic acid and scleroprotein content of the aorta throughout postnatal development and to correlate them with the morphological changes described. Sequential studies of this kind have not been carried out in the rat.

The scope of previous studies on rat aortic nucleic acids is given in Table 1. Results have shown general agreement. Hashimoto & Dayton (1964) found that the total DNA content of the aorta in the rat increased progressively with age. DNA concentration, however, fell progressively, the levels in the adult being about one-fifth of that seen in infancy; this change was attributed to extracellular accumulation of collagen. A later study by Savino *et al.* (1965) showed an increase in both RNA and DNA in the rat aorta as body weight increased, and Stein, Eisenberg & Stein (1969) showed a decrease in DNA concentration in the rat aorta up to 3 months of age, after which it remained constant up to 2 years.

The materials studied in determinations of the scleroprotein content of the aorta of various mammals are shown in Table 2. Kao & McGavack (1959) found the elastin content of the rat aorta was constant at around 6 % of the wet weight in 3–5 week, 8 month and 2 year old animals, while collagen increased from 5 % to 8·1 % of the wet weight. In a study on the effect of hypertension on the structure of the thoracic aorta in adult rats, Wolinsky (1971) found 41·2 g aortic elastin/100 g dry weight and 13·8 g collagen/100 g dry weight for normal males, with higher values for females (44·6 % elastin and 17·8 % collagen).

Harkness, Harkness & McDonald (1957) examined the scleroprotein content of the walls of arteries in the dog. Collagen and elastin together formed 50 % of the dry weight of all arteries studied, except the smallest, where the proportion was somewhat higher. In the intrathoracic aorta there was about twice as much collagen as elastin; in all other vessels this relationship was reversed. The transition between the more elastic intrathoracic aorta and the more collagenous extrathoracic vessel was abrupt.

In general, apart from our own human study (Berry, Looker & Germain, 1972*b*) no sequential examination of changes in nucleic acids and scleroprotein content

of the aorta has been carried out throughout development in any species. No study has related cellularity to scleroprotein content of the vessel wall.

MATERIALS AND METHODS

Wistar rats were maintained as described previously (Berry, Looker & Germain, 1972*a*).

Newborn and 1 week old pups were sacrificed by decapitation and all other animals by chloroform anaesthesia. The entire aorta was removed intact from animals less than 18 days old and the two segments (thoracic and abdominal) removed separately from animals older than this. In both instances the vessel was washed free of blood in saline at 4 °C and placed on saline-soaked filter paper on ice.

Vessels from newborn and 1 week old animals were removed entire, and placed in a Petri dish in saline at 4 °C under a binocular dissecting microscope. All adherent tissue that stripped off easily with forceps was removed (Harkness *et al.* 1957) and branches of the aorta were cut off at their origins. The samples were rinsed in clean cold saline, blotted twice between two sheets of filter paper, and weighed on an Oertling R20 single-pan balance to one-tenth of a milligram. This weight was taken as the wet weight of the tissue. Vessels under 2·5 mg wet weight were pooled for weighing.

For scleroprotein determinations 200 animals were studied in the newborn period, 60 at 1 week, 24 at 18 days and 32 of each sex from 6 to 12 weeks. Eight of each sex were examined at each subsequent determination (12, 18, 22 and 52 weeks). Corresponding figures for nucleic acid determinations were: newborn, 40; 1 week, 24; 18 days, 20; 4 and 6 weeks, 16 of each sex; and 8 of each sex in each group at 8, 12, 18, 22 and 52 weeks.

Samples were wrapped in aluminium foil, placed in capped tubes, labelled and stored in an air-tight container at −20 °C for up to 1 week.

Preparation of tissue for nucleic acid analysis

The arteries were ground to a fine powder in liquid nitrogen with a precooled pestle and mortar. The powder was scraped from all surfaces with a cold microspatula on to aluminium foil lying on solid carbon dioxide, placed in an air-tight container, and stored at −20 °C overnight.

Samples of less than 20 mg wet weight were pooled for analysis. Nucleic acids were separated by the method of Shibko *et al.* (1967) adapted for aortic tissue as described by Looker (1972).

Quantitative determination of RNA

The RNA extracts obtained were diluted with 5 % perchloric acid (PCA) so as to contain approximately 20 μg RNA/ml, and the ultraviolet absorption was subsequently measured at 261 μm against a 5 % blank in 10 mm silica cells using a Pye Unicam SP 500 spectrophotometer. Yeast ribonucleic acid (Type XI Sigma) was used as the standard reference solution in concentrations of 10 to 30 μg/ml, diluted from a stock solution of 100 μg/ml in 0·0005 mM NaOH, stored at 4 °C.

Determination of DNA

DNA was determined by the estimation of deoxypentose according to the diphenylamine technique of Burton (1956), modified by Giles & Meyer (1965), and adapted to this study by Looker (1972).

Preparation for scleroprotein analysis

Samples were partially dried *in vacuo* over phosphorus pentoxide and defatted in ethanol and ether. They were then dried to a constant weight in wide-necked bottles over P_2O_5 *in vacuo*. This measurement is subsequently referred to as dry fat free tissue (DFFT) weight. The tissue was then transferred to 15 ml autoclaving tubes, which were corked and stored at room temperature until the time of analysis.

The minimum amount of wet tissue required for scleroprotein determination was 25 mg. Collagen and elastin were extracted by techniques used by Dr R. D. Harkness and Margaret L. R. Harkness at University College, London, adapted from the methods of Neuman & Logan (1950*a*, *b*) and Lansing *et al.* (1952). Hydroxyproline was determined by Grant's (1964) autoanalyser adaptation of Stegemann's (1958) technique.

Duplicate samples were read at 550 μm against distilled water with 10 μg/ml hydroxyproline (B.D.H.) as a standard reference solution, diluted from a stock solution of 100 μg/ml kept at 4 °C. Cups containing test samples were positioned to alternate with cups containing distilled water. The amount of hydroxyproline in the hydrolysate was converted to its collagen equivalent by multiplication by the factor 7·46 (Neuman & Logan, 1950*b*).

Quantitative estimation of elastin

Elastin was determined gravimetrically, on an Oertling R20 single-pan balance, after treatment of the solid remaining after solubilisation of collagen with hot 0·1 N NaOH for 30 minutes. Amino acid analysis of the extracted elastin from newborn, 6 week, and 22 week aortas was carried out in order to assess its purity.

RESULTS

Aortic wet weight

During the period studied the greatest increment in the wet weight of the entire aorta, in both males and females, occurs between weeks 1 and 8. The rate of increase in wet weight is the same in both sexes until 8 weeks, but after this time weight gain in the female occurs more slowly than in the male. In both sexes the rate of increase in wet weight of the thoracic segment exceeds that of the abdominal. Weight gain in the abdominal aorta is negligible after 22 weeks (Figs. 1, 2).

Water plus lipid content; dry fat free weight

Over the period of time studied the water plus lipid content of the entire aorta falls progressively from 89 % of the wet weight at birth to 70 % at week 22. There is no

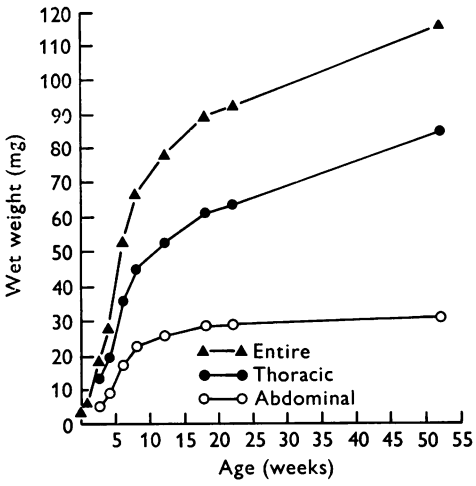

Fig. 1

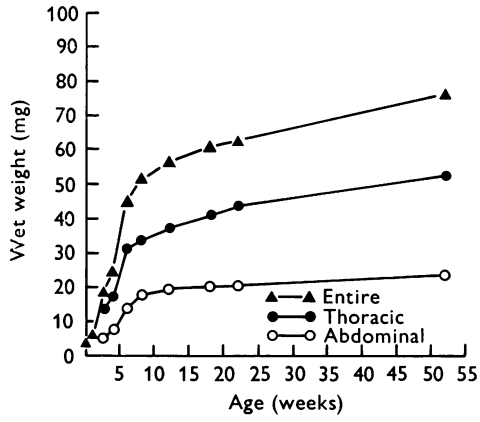

Fig. 2

Fig. 1. Increase in wet weight of the entire, thoracic and abdominal aorta of the male rat with age.

Fig. 2. Increase in wet weight of the entire, thoracic and abdominal aorta of the female rat with age.

difference between male and female during this period, but by 1 year of age the female aorta contains 5 % more water plus lipid than the male. As might be expected, the dry fat free weight increases with age.

DNA content

DNA increases rapidly in the entire aorta of the male rat between weeks 1 and 8. The increase occurs at the same rate in the female between weeks 1 and 6. After this rapid initial increase there is a gradual decrease in the rate of change for the remainder of the time studied. The abdominal aorta attains its maximum DNA content by week 30 while the total DNA in the thoracic segment continues to increase until week 52 (Figs. 3, 4).

The concentration of DNA/unit mass of aorta (mg DNA/100 mg DFFT) for the thoracic and abdominal segments is shown in Fig. 5 for the male rat. Essentially similar curves describe changes in the female, and statistical analysis shows no significant difference between the sexes. There is a 77 % decrease in concentration of DNA during the first 6 weeks of life and a 43 % decrease from week 6 to week 22. The adult level is attained by around week 22 in the female, but in the male the decrease continues for the remainder of the period studied. The abdominal segment has a higher concentration of DNA than the thoracic segment during the period 18 days to 4 weeks ($P < 0{\cdot}001$) but the situation is reversed from shortly after this time (at 6 weeks – male $P < 0{\cdot}02$, female $P < 0{\cdot}01$) until the end of this study. In the male there appears to be little difference between the two segments after week 22.

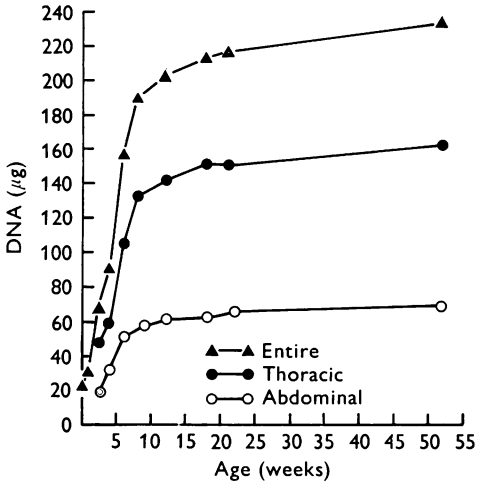

Fig. 3

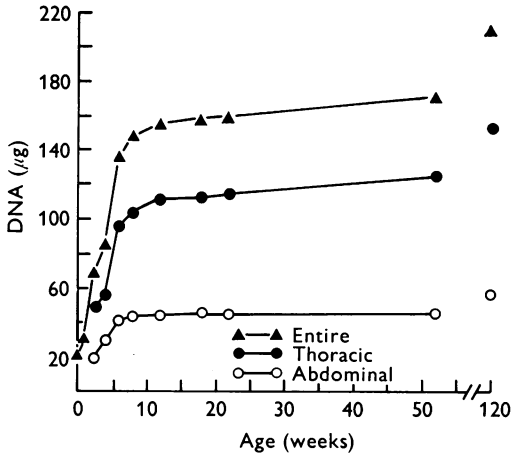

Fig. 4

Fig. 3. Increase in DNA content of the entire, thoracic and abdominal aorta of the male rat with age.

Fig. 4. Increase in DNA content of the entire, thoracic and abdominal segments of the female rat aorta with age.

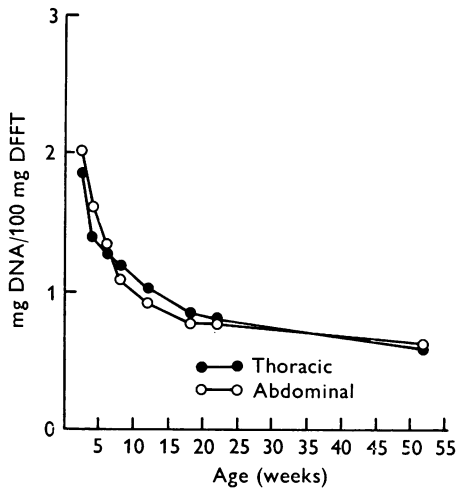

Fig. 5. Change in mg DNA/100 mg DFFT of the thoracic and abdominal segments of the male rat aorta with age.

Cellularity

The number of nuclei in a unit volume of aorta was calculated, assuming a diploid state, and a figure of 6·2 $\mu\mu$g/cell DNA (Enesco & LeBlond, 1962). The results are shown in Fig. 6. Since the values are derived from the DNA content/unit volume of aorta the curves are similar. The entire aorta of the newborn contains approximately

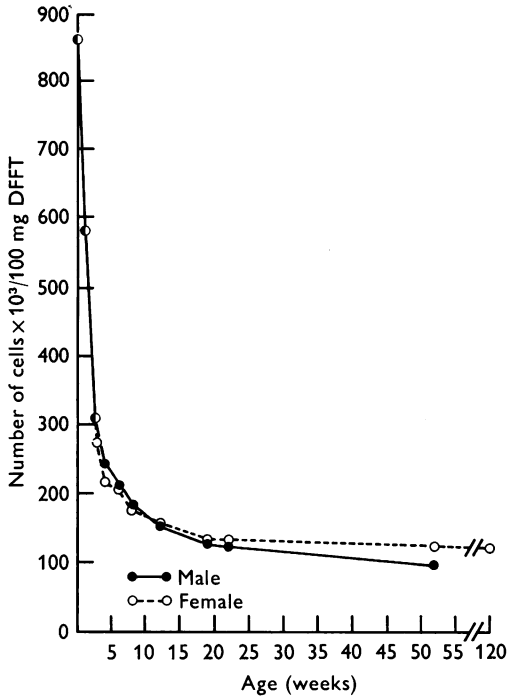

Fig. 6. Decrease in cellularity of the entire aorta with age.

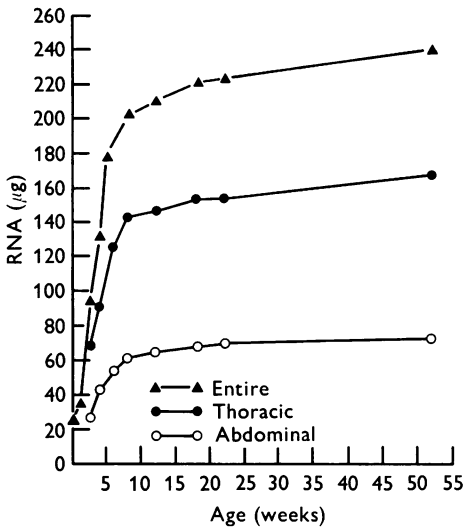

Fig. 7

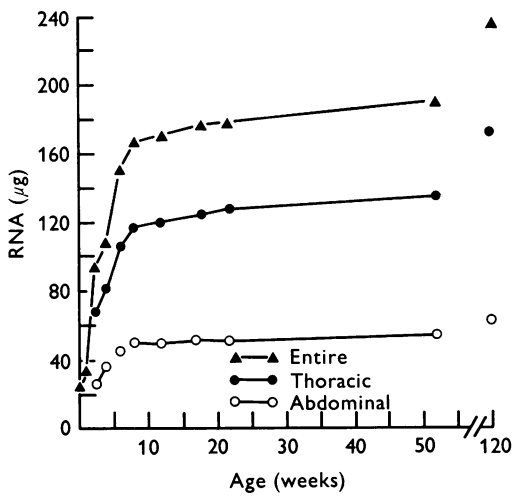

Fig. 8

Fig. 7. Increase in RNA content of the entire, thoracic and abdominal aorta of the male rat with age.

Fig 8. Increase in RNA content of the entire, thoracic and abdominal segments of the female rat aorta with age.

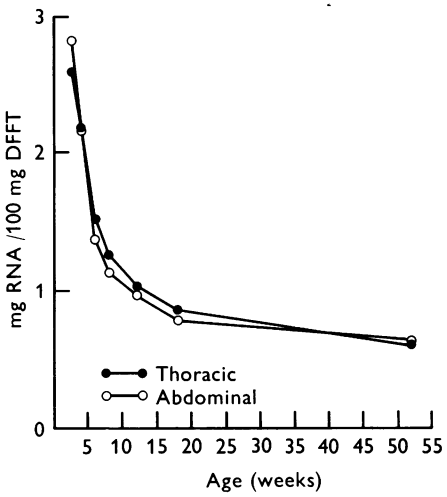

Fig. 9

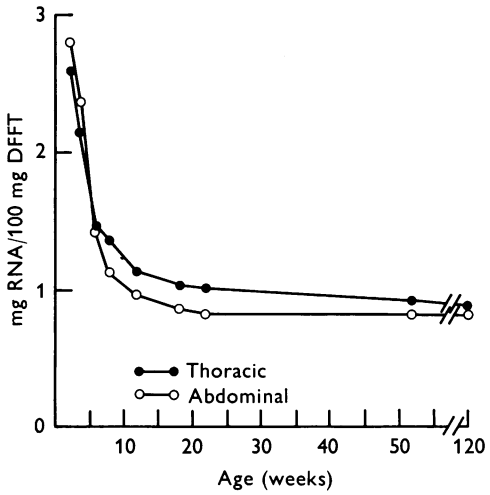

Fig. 10

Fig. 9. Change in mg RNA/100 mg DFFT of the thoracic and abdominal aorta of the male rat with age.

Fig. 10. Change in mg RNA/100 mg DFFT of the thoracic and abdominal segments of the female rat aorta with age.

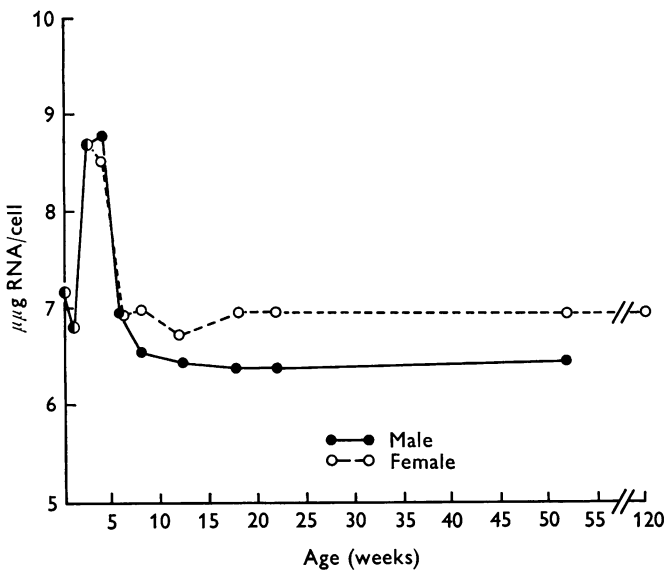

Fig. 11. Change in RNA/cell during development and growth of the entire rat aorta.

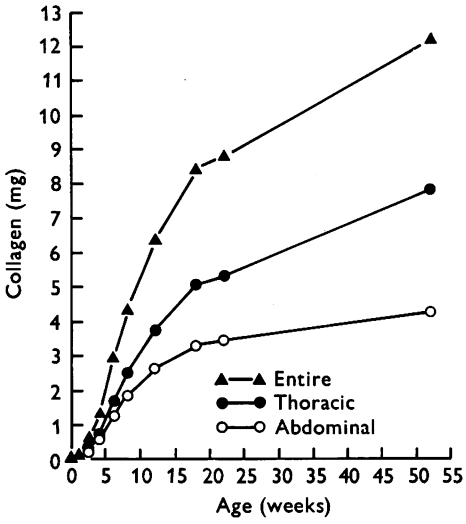

Fig. 12

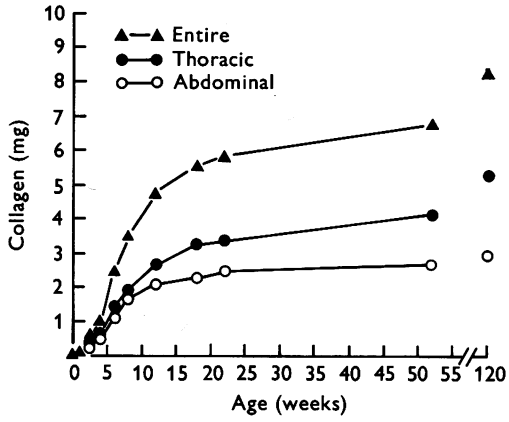

Fig. 13

Fig. 12. Increase in collagen content of the entire, thoracic and abdominal aorta of the male rat with age.

Fig. 13. Increase in collagen content of the entire, thoracic and abdominal aorta of the female rat with age.

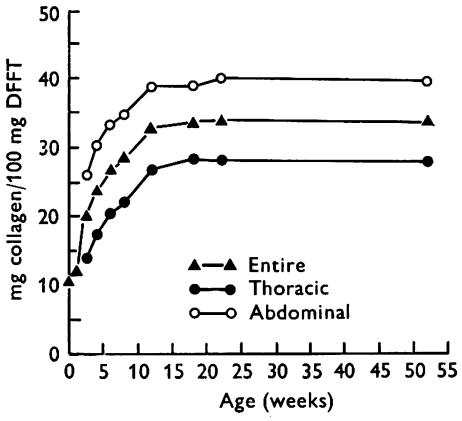

Fig. 14

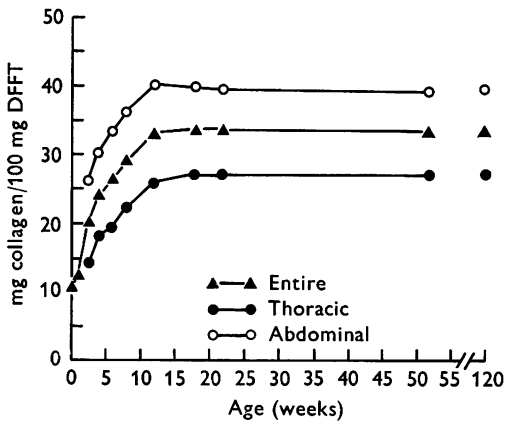

Fig. 15

Fig. 14. Change in mg collagen/100 mg DFFT of the entire, thoracic and abdominal aorta of the male rat with age.

Fig. 15. Change in mg collagen/100 mg DFFT of the entire, thoracic and abdominal aorta of the female rat with age.

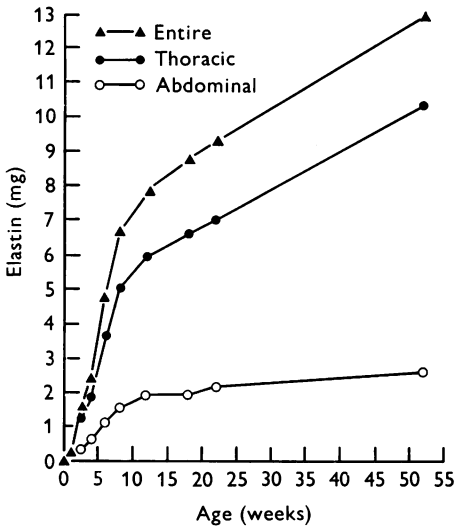

Fig. 16

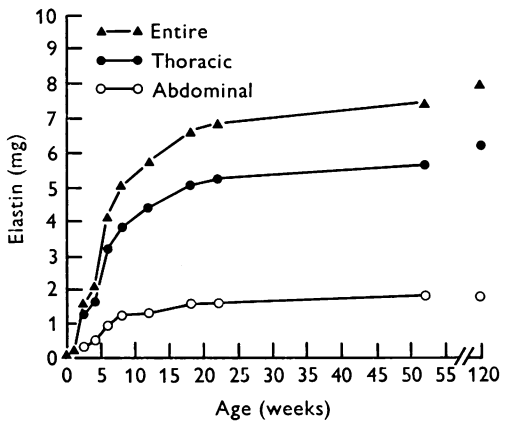

Fig. 17

Fig. 16. Increase in elastin content of the entire, thoracic and abdominal aorta of the male rat with age.

Fig. 17. Increase in elastin content of the entire, thoracic and abdominal aorta of the female rat with age.

8·6 million cells/mg DFFT, whereas the 6 week old vessel contains 2·1 million. At 1 year a milligram of dry fat free male aorta contains 1 million cells and a similar weight of the female aorta 1·2 million cells.

RNA content

The total RNA content of the entire aorta, and for the thoracic and abdominal segments in the male and female, are shown in Figs. 7 and 8. The concentration of RNA per unit volume of aorta (mg RNA / 100 mg DFFT) is shown in Figs. 9 and 10. There is a highly significant difference in RNA concentration between the thoracic and abdominal segments up to 18 weeks in the male, and up to 1 year in the female.

RNA concentration progressively decreases over the period studied, but when expressed as RNA/cell shows a different pattern for the entire vessel with a peak around 18–28 days after birth (Fig. 11). From day 18 until the end of the period studied, the concentration of RNA/cell remains fairly constant at about 6·9 $\mu\mu$g for the female and 6·4 $\mu\mu$g for the male.

Scleroproteins

Collagen

The increase in the amount of collagen is most rapid in the entire vessel in the period 1 week to 18 weeks in the male, and 1 week to 12 weeks in the female (Figs. 12, 13). The rate of increase is similar in both sexes; the thoracic segment contributes more towards this increase than the abdominal segment. After this initial period the

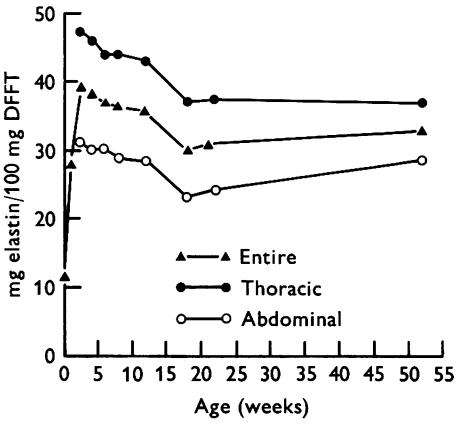

Fig. 18

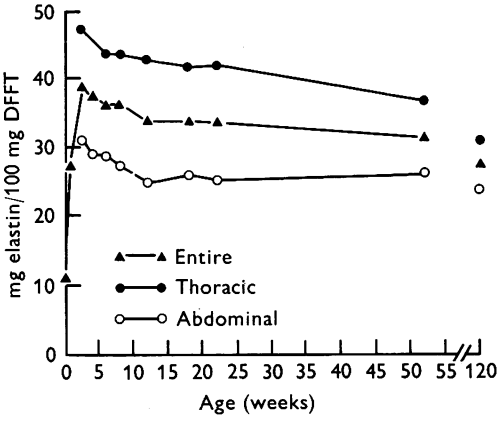

Fig. 19

Fig. 18. Change in mg elastin/100 mg DFFT of the entire, thoracic and abdominal aorta of the male rat with age.

Fig. 19. Change in mg elastin/100 mg DFFT of the entire, thoracic and abdominal aorta of the female rat with age.

rate of increase rapidly diminishes, the rate in the female being about one-third of that in the male. During this 'second phase' the collagen content of the thoracic segment increases by twice as much as the abdominal segment in both male and female.

The amount of collagen in 100 mg DFFT is shown in Figs. 14 and 15 for male and female respectively. There is a rapid increase in collagen concentration in the entire vessel from birth (11 mg/100 mg DFFT) to 12 weeks (33 mg/100 mg DFFT) in both male and female, with the greatest increase occurring between weeks 1 and 4. The thoracic and abdominal segments contribute equally to this change. At 18 days the dry fat free mass of the thoracic aorta is about 14 % collagen. The collagen content is approximately twice this at 12 weeks, when maximum concentration of this component is reached. The dry fat free mass of the abdominal segment is composed of about 26 % collagen at day 18, with a 50 % increase (to 39 % collagen) by 12 weeks.

There is no significant difference between the concentration of collagen in male or female in the entire aorta, or in its thoracic or abdominal segments.

The collagen/cell ratio in 100 mg DFFT of the entire aorta and in the thoracic and abdominal segments is shown in Table 4 for male and female. In the entire aorta there is a gradual increase in the amount of collagen per cell with age, and the abdominal segment has a considerably higher collagen/cell ratio than the thoracic segment. Until 22 weeks the male thoracic aorta has a greater collagen/cell ratio than the female; the converse is true until week 18 for the abdominal segment. After this time the ratio is greater in the male in both segments.

Elastin

The most rapid increase of total alkali-insoluble elastin in the entire aorta of both male and female occurs between weeks 1 and 18. The rate of increase during this

Table 1. *Nucleic acid determinations on normal rat aorta*

Authors	Aortic segment	Part of wall	Sex	Age	Nucleic acid estimated
Priest (1962)	Proximal and distal	ns	ns	Adult	DNA
Gerschenson *et al.* (1962)	Entire	I+M	Male and female	Adult	DNA, RNA
Hashimoto & Dayton (1964)	Entire	I+M	Male	5–232 days	DNA
Savino *et al.* (1965)	Entire	I+M	Male	30–170 days	DNA, RNA
Wortman *et al.* (1966)	Entire	I+M	Male	'Weanlings' and animals 1 month older	DNA, RNA
Stein *et al.* (1969)	Entire	I+M	Male	1 month–2 years	DNA

Key: ns = not specified; I = intima; M = media.

Table 2. *Scleroprotein determinations on normal mammalian aorta*

Authors	Species	Aortic segment Examined	Part of Wall	Age (weeks)	Scleroprotein estimated
Neuman & Logan (1950*b*)	Rat, pig, bovine	Arch	ns	ns	C, E
Harkness *et al.* (1957)	Dog	Multiple, along vessel	I+M	0–6	C, E
Kao & McGavack (1959)	Rat	ns	ns	0–32	C, E
Kao *et al.* (1960)	Rat	ns	ns	4–130	C
Kao *et al.* (1961)	Rat	ns	ns	5–18	C, E
Lorenzen (1961)	Rabbit	Arch, thoracic, abdominal	I+M	Adult	C, E
Cleary (1963)	Many, including giraffe and sheep	Multiple, along vessel	I+M	Adult	C, E
Grant (1967)	Pig, sheep, goat, man	Multiple, along vessel	I+M	Wide range	C, E
Wolinsky & Glagov (1969)	Rat	Entire vessel	M	Adult	C, E
Wolinsky & Daly (1970)	Rat, rabbit	Entire vessel	I+M	Adult	C, E
Wolinsky (1971)	Rat	Entire vessel	I+M	Adult	C, E

Key: ns = not specified; I = intima; M = media; C = collagen; E = elastin.

period is the same in both sexes, but subsequently the rate of increase in the male is greater than that in the female for the remainder of the period studied. The pattern of increase in elastin in the thoracic and abdominal segments is essentially as described for the entire vessel. Increase in the elastin content of the thoracic segment is considerably greater than in that of the abdominal segment; this effect is more marked in the male (Figs. 16, 17).

The concentration of elastin (mg elastin/100 mg DFFT) of the entire and the thoracic and abdominal segments of the aorta is shown in Figs. 18 and 19 for male and female respectively. There is a considerable increase in elastin from birth to 18 days in the entire vessel of both sexes, from 11 mg/100 mg DFFT to 39 mg/100 mg

Table 3. *Collagen (μμg) and elastin (μμg) per cell in 100 mg DFFT of the entire, thoracic and abdominal rat aorta*

Sex	Age (weeks)	Collagen/cell (μμg)			Elastin/cell (μμg)		
		Thoracic	Abdominal	Entire	Thoracic	Abdominal	Entire
Pooled samples	0	—	—	13	—	—	13
	1	—	—	21	—	—	48
	2·6	47	80	64	159	96	126
Male	4	78	117	99	206	116	157
	6	100	155	128	213	140	175
	8	116	199	156	231	165	199
	12	164	262	211	261	192	228
	18	211	317	261	273	187	232
	22	219	324	270	291	195	244
	52	294	397	346	389	286	336
Female	4	73	101	88	186	98	138
	6	92	163	127	207	141	175
	8	113	227	164	226	173	208
	12	154	281	212	256	176	219
	18	179	330	246	279	216	251
	22	184	334	252	287	212	254
	52	200	347	266	272	234	255
	117	214	343	276	245	208	227

Table 4. *Change in total scleroprotein (mg/100 mg DFFT) of the entire, thoracic and abdominal segments of the rat aorta with age*

Sex	Age (weeks)	Thoracic	Abdominal	Entire
Pooled samples	0	—	—	22·1
	1	—	—	40·2
	2·6	61·3	57·4	59·3
Male	4	63·6	60·6	62·1
	6	64·6	63·7	64·2
	8	66·6	64·0	65·1
	12	70·0	67·5	68·7
	18	65·5	62·0	63·8
	22	65·6	64·2	64·9
	52	65·1	68·1	66·6
Female	4	64·1	59·6	61·9
	6	63·6	62·5	63·0
	8	66·2	63·6	64·9
	12	69·2	65·0	67·0
	18	69·1	66·2	67·6
	22	69·1	65·4	67·2
	52	64·3	65·5	64·8
	117	58·3	63·8	61·0

Table 5. *Amino acid composition of rat aortic elastin*

Amino acid	Age		Bentley & Hanson (1969)
	Newborn	Six weeks	
Aspartic acid	11·11	7·28	4·45
*Threonine	13·62	14·41	7·05
*Serine	14·82	11·99	8·25
Glutamic acid	22·06	16·74	15·55
Proline	113·61	115·47	104·50
Glycine	340·59	360·79	353·50
Alanine	206·26	204·07	218·00
Valine	86·71	101·00	105·50
Methionine	1·58	0·62	0·90
Isoleucine	27·80	26·17	27·50
Leucine	70·25	66·38	68·00
Tyrosine	41·33	34·15	29·50
Phenylalanine	25·79	27·38	16·00
Histidine	3·50	2·37	0·95
Lysine	11·24	3·19	4·05
Arginine	9·72	8·16	13·35
Cystine	0·00	0·00	0·00

Results expressed as residues per total 1000 residues.
* Not corrected for degradation.

DFFT. After 18 days a progressive decrease in the concentration is found, to about 33 mg/100 mg DFFT at 22 weeks. The thoracic aorta is richer in elastin than the abdominal aorta; for example, in the 18 day female the thoracic segment is composed of 47 % elastin while the abdominal part contains 31 % elastin.

The elastin/cell ratio found for 100 mg DFFT of the entire, thoracic and abdominal aorta is presented in Table 3 for both male and female. There is a gradual increase in the ratio with increasing age. The thoracic segment has a higher elastin/cell ratio than the abdominal. There is no apparent difference between male and female until 1 year, when the male aortic segments have considerably more elastin per cell than those of the female.

The total scleroprotein content of the rat aorta is shown in Table 4. Together collagen and elastin make up approximately 22 % of the dry fat free weight of the entire aorta of a newborn rat, and approximately 70 % in the adult rat.

The amino acid composition of the rat 'elastin' samples are shown in Table 5. The results indicate that there is very little protein contamination of our extract. Tyrosine accounts for only 3–4 % of the rat elastin residues; the large numbers of glycine, proline and valine residues indicate that these samples are unquestionably 'elastin'.

The results of amino acid analysis of rat aortic elastin carried out by Bentley & Hanson (1969) are shown in Table 5 for comparison.

DISCUSSION

The wet weight of the aorta increases most rapidly in the first 8 weeks of postnatal life in both sexes, although the period of rapid growth in terms of body weight and crown–rump length is several weeks longer than this, particularly in males (Berry, Looker & Germain, 1972*a*). Aortic length increases most rapidly between weeks 1 and 8, whilst light microscopy shows that the wall thickness increases during the first 6 weeks of extrauterine life, after which little change is seen. Initially, therefore, increases in both wall thickness and aortic length contribute towards the increase in wet weight; subsequent change is apparently due to increase in length of the vessel alone.

DNA gain shows a similar pattern of change to that found for the wet weight, tending to slow down rather sooner in the female aorta, in both the thoracic and abdominal segments. In both sexes the gain in DNA in the thoracic segment continues until the end of the period studied. The values for total DNA content of the entire male rat aorta described by Hashimoto & Dayton (1964) are in good general agreement with those found in this study until week 4, but after this time our values are higher. We attribute this difference to the improved extraction procedure used; after 4 weeks the aorta is more fibrous and cells may remain trapped between lamellae after homogenization. Hashimoto & Dayton defined the age of the vessel by change in wet weight, which makes direct comparison of results difficult. Savino *et al.* (1965) showed that ageing in rats is associated with a higher concentration of nucleic acids in the aorta. Hashimoto & Dayton's study (1964) and our own work has shown that nucleic acid concentration decreases with age. Savino and his co-workers ascribed the increase in nucleic acid they found to the accumulation of lymphocytes in the tissue of older animals, noting that the greatest change in the number of lymphocytes was in the adventitia. Since they stripped this layer before analysis, as we have done, it seems unlikely that lymphocyte infiltration explains their results. A more probable source of error is their extraction of nucleic acids by the Ogur and Rosen method, which has proved inadequate for determining nucleic acids in biological material (Hutchison & Munro, 1961).

There is a progressive decrease in DNA concentration in the growing rat aorta, and thus the cell number per unit volume must fall. From birth to 6 weeks 77% reduction in cellularity occurs, and a further 43% fall occurs between 6 and 22 weeks, the change being obvious on microscopy (Berry *et al.* 1972*a*). The rate of change is the same for both sexes until week 16, at which time the slower rate of decrease in DNA concentration in the female is evident. This change may be due to the effects of progesterone and oestrogen on smooth muscle cells, since it is about this time that the female attains sexual maturity. Recently Wolinsky (1972) has shown that these sex hormones affect vascular, as well as genito-urinary, smooth muscle. Nevertheless, the rapid increase in scleroprotein concentration during the first 6 weeks of postnatal life accounts for much of the decrease in concentration of DNA during this period.

The concentration of DNA is greater in the thoracic than in the abdominal aorta in the adult animal. Priest (1962) showed that in the adult the proximal aorta is composed of 0·29% DNA, whereas the distal aorta contains only 0·21%. He also

demonstrated that the oxygen consumption of the proximal aorta is twice that of the distal part, suggesting a greater cellular activity in this segment. Our findings agree with his; at 12 weeks the thoracic and abdominal segments contain approximately 0·285% and 0·235% DNA respectively, a significant difference which is not found in early development. From 18 days to 6 weeks the abdominal aorta contains more DNA per unit volume of tissue than the thoracic segment. The difference is significant at 18 days but becomes progressively less so until week 6, when the concentration in each segment is similar. This observation supports the view that in the early weeks of life the processes of cell division and elastin and collagen synthesis are more active in the abdominal segment than in the thoracic, and also Fyfe, Gillman & Oneson's (1968) observation of a 'wave of elastification' travelling down the vessel. If this is so, individual analysis of thoracic and abdominal segments before 18 days may show that the thoracic aorta is richer in DNA, but because of the difficulty in obtaining sufficient tissue we have not been able to demonstrate this. From every aspect the abdominal segment apparently obtains its 'adult' or 'final' structure considerably earlier than the thoracic aorta, which continues to change in composition throughout the period studied.

The differences between the thoracic and abdominal segments in RNA content and concentration are similar to those described for DNA, supporting the view that for some weeks after birth the abdominal aorta is more metabolically active than the thoracic segment. In the adult the concentration of RNA is greater in the thoracic aorta, the change occurring at around 6 weeks. The striking peak in RNA concentration per cell between 18 and 28 days is presumably related to the intense metabolic activity in the vessel wall at this time. A similar peak of metabolic activity is seen in the human aorta during the mid trimester (Berry *et al.* 1972*b*).

Collagen and elastin both increase rapidly from 1 to 18 weeks in the male, and from 1 to 12 weeks in the female, although the total cell number per aorta is almost maximal by 6 weeks. The rapid increase in collagen and alkali insoluble elastin continues after this, and subsequently scleroprotein deposition is maintained for some time. The observations of Cliff (1967) suggest that the aortic smooth muscle cell changes from a synthetic to a synthetic and contractile, and finally to a mainly contractile, cell around 3 months of age. Since the smooth muscle cell is the only cell type found in the media, and since scleroprotein deposition continues well after week 12, it would appear that in the 'predominantly contractile' celled aorta some cells do synthesise considerable amounts of both collagen and elastin. The stimulus for scleroprotein synthesis in the aortic wall is thought to be change in intramural tension. In early life in the rat, when blood pressure increases progressively, the number of elastic lamellar units increases. From our morphological observations (Berry *et al.* 1972*a*) it appears that at first the bulk of elastic tissue synthesis is directed towards formation of these elastic lamellae. This is completed by around week 5 in both the thoracic and abdominal segments. After this time elastic tissue deposition contributes to an increase in lamellar thickness and compaction of elastic tissue within the laminae, and to the formation and development of branched lamellae. Simultaneously increased scleroprotein synthesis is necessary as the vessel increases in length. The rates of production of collagen and elastin in the thoracic and abdominal segments change both absolutely and in relation to one another. At

18 days elastin represents about 47% of the dry fat free weight of the thoracic aorta and 32% of the abdominal segment; by week 12 this has decreased to about 44% and 27% for thoracic and abdominal segments respectively. At 18 days collagen represents about 14% of the dry fat free weight of the thoracic aorta and 26% of the abdominal segment; by week 12 this has risen to 25% and 39% for thoracic and abdominal segments respectively. The entire vessel is predominantly elastic until week 12, after which similar concentrations of collagen and elastin are present. The relative proportions of collagen and elastin in the thoracic and abdominal segments of the mammalian aorta differ markedly, as pointed out by Harkness *et al.* (1957) and Cleary (1963). These authors found that elastin predominates in the thoracic and collagen in the abdominal segment. We have found that whilst 'elastin predominance' is found in the thoracic segment throughout development, the abdominal aorta at 18 days and 4 weeks contains more elastin than collagen – the adult 'collagen predominance' situation being established later.

There is a very rapid increase in the concentration of alkali-insoluble elastin in the entire aorta, from about 11% of the dry fat free mass at birth to nearly 40% at 18 days. These values almost certainly do not represent the total elastin content of the vessel, but only the cross-linked fraction – i.e. the mature elastin (see below). An apparent decrease in the concentration of elastin in the entire vessel is found between 18 days and 18 weeks, after which the concentration remains constant. This 'decrease' is due to the rapid increase in the concentration of collagen from approximately 11% at birth to 33% at 18 weeks, after which it remains constant for the remainder of the period studied. The increase in absolute amounts of collagen and elastin after 18 weeks of age is more marked in the male, but changes in concentration are similar in the two sexes. The concentration of total scleroprotein in the entire aorta increases from 22% at birth to about 65% in the adult. The total scleroprotein content of the thoracic and abdominal parts of the aorta is comparable, and there is no significant difference between male and female. The collagen values found in our study are comparable to those obtained by Kao & McGavack (1959) for the entire aorta of the female rat at 4, 32 and 104 weeks of age. Their elastin values from similar vessels are considerably lower than ours, although similar extraction procedures were used. The difference may be explained by differing selection of tissue for extraction, since they do not state which part of the aorta was examined.

The change with age in collagen and elastin per cell in a unit volume of aorta clearly demonstrates the change in the vessel wall, from a cellular structure at birth to a more densely elastic and collagenous vessel in the adult. As expected the collagen/cell ratio is higher in the thoracic segment. At 1 year of age thoracic and abdominal segments of the female aorta have considerably less collagen and elastin per cell than the male.

Amino acid analysis of rat aortic elastin in this study was carried out to assess the purity of the extracted material. Table 5 shows that rat aortic elastin is composed of 1·1% lysine at birth and 0·32% lysine at 6 weeks of age, indicating that elastic tissue synthesis is more rapid in the newborn than in the 6 week old vessel. The lysine content of elastin during periods of rapid synthesis is higher than that found during slower elastin formation since cross-linking in elastin takes place by conversion of

four lysine residues to desmosine and isodesmosine through a series of intermediate steps (Franzblau & Lent, 1968). Our results agree well with those of Bentley & Hanson (1969). Slight differences are found, and these authors estimated several amino acids not analysed in our study, but included in their estimate of residues per 1000 residues. Since the purpose of amino acid analysis in this study was to establish the nature and purity of the hot alkali extracted elastic tissue, these differences were not considered further.

In summary, it is evident that the rat aorta continues to grow and develop well into adult life, and that its chemical constituents and histological form change with age. These findings may be practically useful in dissociating age-related phenomena from degenerative changes. 'Late' development of the aorta occurs in man and other species and studies relating modification of structure by dynamic stresses in growing vessels may give useful information concerning the changes in arterial form during growth.

SUMMARY

Extensive changes in the nucleic acid and scleroprotein content of the rat aorta occur during development. In general, levels of DNA and RNA change little after twelve weeks of age; absolute amounts of collagen and elastin increase throughout the first 30 weeks of life in the female and the first year in the male. Sex differences in concentration of components are established by about 16 weeks. Considerable differences are found between thoracic and abdominal segments.

This work is supported by the British Heart Foundation.

REFERENCES

BENTLEY, J. P. & HANSON, A. N. (1969). The hydroxyproline of elastin. *Biochimica et biophysica Acta* **175**, 338–344.

BERRY, C. L., LOOKER, T. & GERMAIN, JOCELYN (1972*a*). The growth and development of the rat aorta. I. Morphological aspects. *Journal of Anatomy* **113**, 1–16.

BERRY, C. L., LOOKER, T. & GERMAIN, JOCELYN (1972*b*). Nucleic acid and scleroprotein content of the developing human aorta. *Journal of Pathology* (in Press).

BURTON, K. (1956). A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochemical Journal* **62**, 315–323.

CLEARY, E. G. (1963). A Correlative and Comparative Study of the Non-uniform Arterial Wall. Ph.D. Thesis, University of Sydney.

CLIFF, W. J. (1967). The aortic tunica media in growing rats studied with the electron microscope. *Laboratory Investigation* **17**, 599–615.

ENESCO, M. & LEBLOND, C. P. (1962). Increase in cell number as a factor in the growth of the organs and tissues of the young male rat. *Journal of Embryology and Experimental Morphology* **10**, 530.

FRANZBLAU, C. & LENT, R. W. (1968). Studies on the chemistry of elastin. *Brookhaven Symposia in Biology Number* 21: *part* 2, *Structure and Evolution in Proteins*, pp. 358–377. Upton, N.Y.: Brookhaven National Laboratory.

FYFE, F. W., GILLMAN, T. & ONESON, I. B. (1968). A combined quantitative chemical, light and electron microscope study of aortic development in normal and nitrite treated mice. In: Atherosclerosis: recent advances. *Annals of the New York Academy of Sciences* **149**, 591–627.

GERSCHENSON, L., MALINOW, M. R., LACUARA, J. L. & MOGUILEVSKY, H. C. (1962). Changes in the aortic concentration of nucleic acids induced by gonadectomy in rats. *Journal of Atherosclerosis Research* **2**, 365–372.

GILES, K. W. & MEYER, A. (1965). An improved diphenylamine method for the estimation of deoxyribonucleic acid. *Nature, London* **206**, 93.

GRANT, R. A. (1964). Estimation of hydroxyproline by autoanalyser. *Journal of Clinical Pathology* **17**, 685–686.

GRANT, R. A. (1967). Content and distribution of aortic collagen, elastin and carbohydrate in different species. *Journal of Atherosclerosis Research* **7**, 463–472.

HARKNESS, M. L. R., HARKNESS, R. D. & MCDONALD, D. A. (1957). The collagen and elastin content of the arterial wall in the dog. *Proceedings of the Royal Society of London, Series* B **146**, 541–551.

HASHIMOTO, S. & DAYTON, S. (1964). Changes in cellularity of normal aorta during maturation. *Journal of Atherosclerosis Research* **4**, 258–260.

HUTCHISON, W. C. & MUNRO, H. N. (1961). The determination of nucleic acids in biological materials. *Analyst* **86**, 768–813.

KAO, K-Y. T. & MCGAVACK, T. H. (1959). Connective tissue. I. Age and sex influence on protein composition of rat tissues. *Proceedings of the Society of Experimental Biology and Medicine* **101**, 153–156.

KAO, K-Y. T., HILKER, D. M. & MCGAVACK, T. H. (1960). Connective tissue. III. Collagen and hexosamine content of the tissues of rats of different ages. *Proceedings of the Society of Experimental Biology and Medicine* **104**, 359–361.

KAO, K-Y. T., HILKER, D. M. & MCGAVACK, T. H. (1961). Connective tissue. IV. Synthesis and turnover of proteins in tissues of rats. *Proceedings of the Society of Experimental Biology and Medicine* **106**, 121–124.

LANSING, A. I., ROSENTHAL, T. B., ALEX, M. & DEMPSEY, E. W. (1952). The structure and chemical characterisation of elastin fibres as revealed by elastase and electron microscopy. *Anatomical Record* **114**, 555.

LOOKER, T. (1972). A Biochemical and Morphological Study of the Development and Growth of the Aorta in the Rat and in Man, with particular reference to Nucleic Acids and Scleroproteins. Ph.D. Thesis, University of London.

LORENZEN, I. (1961). Vascular tissue under the influence of thyroxine. II. Effects of 1-thyroxine on the aortic wall in rabbits. *Acta endocrinologica, Copenhagen* **37**, 183–190.

NEUMAN, R. E. & LOGAN, M. A. (1950*a*). The determination of hydroxyproline. *Journal of Biological Chemistry* **184**, 229–306.

NEUMAN, R. E. & LOGAN, M. A. (1950*b*). The determination of collagen and elastin in tissues. *Journal of Biological Chemistry* **186**, 549–556.

PRIEST, R. E. (1962). Differences in metabolism of proximal and distal aorta. *Circulation* **26**, 667.

SAVINO, E. A., MALINOW, M. R., GERCHENSON, H. C., MOGUILEVESKY, H. C., MARUFFO, C. A., DEPAOLI, J. R. & BALDASSARRE, A. (1965). Changes in the concentration of nucleic acids in the aorta of rats in relation to body weight. *Journal of Atherosclerosis Research* **5**, 397–402.

SHIBKO, S., KOIVISTOINEN, P., TRATNYEK, C. A., NEWHALL, A. R. & FRIEDMAN, L. (1967). Method for the sequential quantitative separation and determination of protein, RNA, DNA, lipid and glycogen from a single rat liver homogenate, or from a subcellular fraction. *Analytical Biochemistry* **19**, 514–528.

STEGEMANN, H. (1958). Mikrobestimmung von Hydroxyprolin mit Chloramin-T und p-Dimethylaminobenzaldehyd. *Zeitschrift für physiologische Chemie* **311**, 41–45.

STEIN, O., EISENBERG, S. & STEIN, Y. (1969). Aging of aortic smooth muscle cells in rats and rabbits. A morphologic and biochemical study. *Laboratory Investigation* **21**, 386–397.

WOLINSKY, H. (1971). Effects of hypertension and its reversal on the thoracic aorta of male and female rats (morphological and chemical studies). *Circulation Research* **28**, 622–637.

WOLINSKY, H. (1972). Effects of oestrogen and progestogen treatment on the response of the male rat to hypertension. *Circulation Research* **30**, 341–349.

WOLINSKY, H. & DALY, M. M. (1970). Method for the isolation of intima media samples from arteries. *Proceedings of the Society of Experimental Biology and Medicine* **135**, 364–368.

WOLINSKY, H. & GLAGOV, S. (1969). Comparison of abdominal and thoracic aortic media structure in mammals. *Circulation Research* **25**, 677–686.

WORTMAN, B., LEE, K. T., KIM, D. N., DAOUD, A. S. & THOMAS, W. A. (1966). DNA, RNA and protein in aortas of rats fed athergenic diets for one month. *Experimental and Molecular Pathology* Suppl. **3**, 88–95.