11 Mulligan, R. C., and Berg, P., Experession of a bacterial gene in mammalian cells. Science *209* (1980) 1422–1427.
12 Neumann, E., Schaefer-Ridder, M., Wang, Y., and Hofschneider, P. H., Gene transfer into mouse L-cells by electroporation in high electric fields. EMBO J. *1* (1982) 841–845.
13 Palmiter, R. D., and Brinster, R. L., Transgenic mice. Cell *41* (1985) 343–345.
14 Parkman, R., The application of bone marrow transplantation to the treatment of genetic diseases. Science *232* (1986) 1374–1378.
15 Pellicer, A., Wigler, M., Axel, R., and Silverstein, S., The transfer and stable integration of the HSV thymidine kinase gene into mouse cells. Cell *14* (1979) 133–141.
16 Proceedings of the III. World Congress of in vitro Fertilization and Embryo Transfer, Helsinki, Finland (1984).
17 Schaffner, W., Direct transfer of cloned genes from bacteria to mammalian cells. Proc. natn. Acad. Sci. USA (1980) 2163–2167.
18 Shapiro, L. J., Treatment of genetic diseases, in: Principles and Practice of Medical Genetics, pp. 1488–1496. Eds A. E. H. Emery and D. Rimoin. Churchill Livingstone, Edinburgh and London 1983.
19 Spradling, A. C., and Rubin, G. M., The effecxt of chromosomal position on the expression of the drosophila xanthine dehydrogenase gene. Cell *34* (1983) 47–57.
20 Williams, D. A., Orkin, S. H., and Mulligan, R. C., Retrovirus-mediated transfer of human adenosine deaminase sequences into cells in culture into murine hematopoietic cell vivo. Proc. natn. Acad. Sci. USA *83* (1986) 2566–2570.

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Full Papers

Changes in pulse wave velocity with age in man: a longitudinal series over 20 years

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Summary. Measurements of pulse wave velocity (PWV) carried out systematically in the same 60 healthy individuals over 20 years confirmed that average PWV progressively increases with age, and more rapidly after age 45. The PWV increase mainly results from the decreased extensibility of the thoracic aorta in the aortic-popliteat arterial system. This longitudinal study showed that PWVs of most individuals do not closely follow the average cross-sectional trend, but vary considerably in type. Therefore, PWV by itself is hardly a close correlate of an individual's physiological age or life expectancy. Individuals with a constantly high PWV or a late abrupt increase are exceptional; a possible relation to an increased mean arterial pressure and decreased arterial extensibility as in hypertonia is mentioned.
Key words. Pulse wave velocity; longitudinal study.

In honor of Fritz Verzár (1886–1979) who, as an all-round physiologist and a pioneer in molecular biology and experimental gerontology, devoted his life to interdisciplinary research.

This physiological investigation into aging was initiated in 1954/55 by Fritz Verzár, then Professor of Physiology at Basel University. As Verzár's successor, I continued this investigation for another 20 years, until 1976. My contribution was part of the teamwork carried out by the Basel Interdisciplinary Longitudinal Study, in association with my colleagues O. Gsell (Internal Medicine), R. Brückner (Ophthalmology) and E. Batschelet (Statistics). A first paper on age-related changes of pulse wave velocity (PWV) in man was published after the first ten years of the study[1]. The present report covers all PWV measurements over 20 years in 60 healthy male probands. During the last 10 years, our team submitted the large volume of data to statistical computer analysis (F. Gutzwiller and F. Hugenschmidt).
Previous cross-sectional studies have shown that PWV increases with age because of hardening of the arterial wall. Since PWV increases steadily with age in each individual, it might be a useful measure of physiological age. Furthermore, it might contribute to a better understanding of the physical and chemical conditions responsible for alterations of the arterial wall in physiological and pathological aging.

Methods

Measurement of PWV. We selected 60 healthy men who each contributed 5 to 6 measurements, distributed as evenly as possible over at least 15 years. Most of these volunteers, staff members of a Basel pharmaceutical company, were able to produce PWV data over a period of 20 years. Every year, measurements of PWV were taken during the same period (September/October) and at the same time of day (09.00–10.00 h) in order to avoid differences due to possible seasonal or circadian influences. Originally, PWV measurements were carried out once a year in a few probands and every second year in most of the subjects. The experiment ended in October 1976.
This long-term study was made possible by the stability of the group of probands, who remained for a long time in the same city.
PWV was measured by the method of Boucke and Brecht[2]. It is determined in the arterial circulation between the aorta (near the heart) and the foot (the arteria dorsalis pedis) in the prone position (fig. 1). On the left foot the pulse wave on the a. dors. pedis (A″ in fig. 1) was

recorded with an infrasonic device and an electrocardiograph (Schwarzer C III) together with the R-wave just preceding the left ventricle systole. The peak of the R-wave and the onset of the pulse wave in the sphygmogram of the a. dors. pedis was measured. A reliable average value was obtained from 10 determinations per proband, and was converted into seconds on the basis of the paper transport velocity (25 mm/s) (example in fig 1. 0.29 s). Pathlength was measured as the distance between the left sterno-clavicular joint close to the aorta and the recording site on the left foot (example in fig. 1: distance $A' - A'' = 1.40$ m). The velocity of the pulse wave (PWV) was calculated as V = distance in m/time in seconds (example in fig. 1: 4.8 m/s).

The method of Boucke and Brecht[2] gives a rough approximation, but one that is acceptable in this context because a standardized technique was used. The 'transit' time measured by these authors includes the time between the R-peak and the beginning of left ventricular ejection, which is about 0.06 s or one-fifth of the total measured time, and the vertical distance measured is not the same as the path-length through the arteries. Both of these variables bear a direct and probably constant relation to the actual transit time and path length, however, and they are a reasonable, practical compromise.

Results

Cross-sectional study. In cross-sectional analysis, we plot the individual data at the beginning of the longitudinal study in a coordinate system of PWV versus age (fig. 2), one value per proband. In figure 2 data from 60 probands are given. The age-dependency is obvious. We have drawn a line representing the average trend (median line) by using medians of PWV for subsequent 5-year age groups and then smoothing the medians (moving average). To estimate dispersion, we have plotted the standard deviation around the median (dashed lines in fig. 2). The area betwen ±1 SD contains roughly two-thirds of the total data points. The median rises and the standard deviation increases with age, this increase being steeper above the age of 50. Table 1 lists the numerical data for the full decades. We can see that PWV increases by 0.16 m/s from age 20 to 30, but by 0.45 m/s from age 50 to 60.

Longitudinal study. Individual behavior of PWV with increasing proband age is shown in figures 3A to 3L. We have plotted 41 of the 60 available diagrams. Each individual proband is represented by a dashed line extending over 15 to 20 years. The median of the cross-sectional plot is also included in each group of lines. The identification numbers of the probands are added. One can see that individual trends do not always closely follow the median trend. Indeed, only a few individuals exhibit a PWV line fairly close to the median line (No. 17 in fig. 3A, No. 112 in fig. 3C, No. 20 in fig. 3D, etc.). Most individual PWV lines deviate considerably from the average cross-sectional trend. There are individuals with higher than average PWV lines (No. 76 in fig. 3B, No. 37 and 58 in fig. 3C, etc.) and others with lower than average lines (No. 108 in fig. 3A, No. 4 and 103 in fig. 3B). Furthermore, we find individuals whose PWV line rises more rapidly than the median line, which may indicate a rela-

Table 1. Median pulse wave velocity in 5 age groups. Transversal (cross-sectional data taken at the beginning of the longitudinal study.

Age	Median PWV (m/s)	SD
20	4.72	0.40
30	4.88	0.47
40	5.07	0.55
50	5.37	0.63
60	5.82	0.73

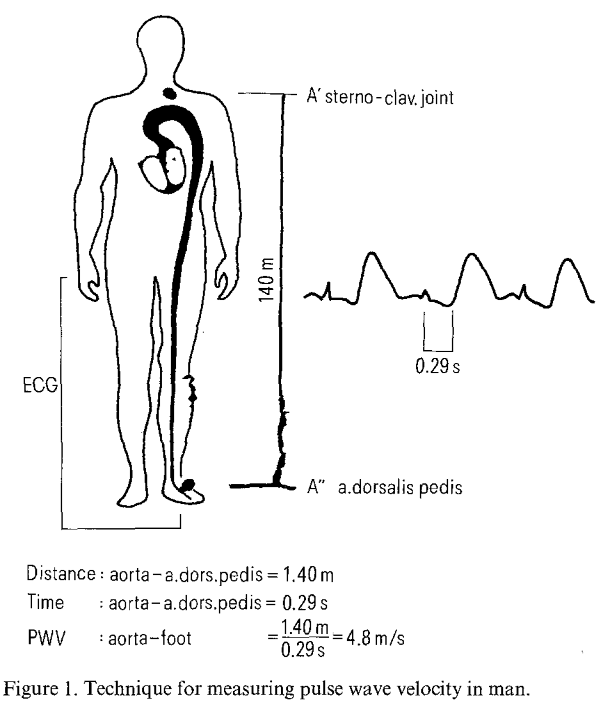

Figure 1. Technique for measuring pulse wave velocity in man.

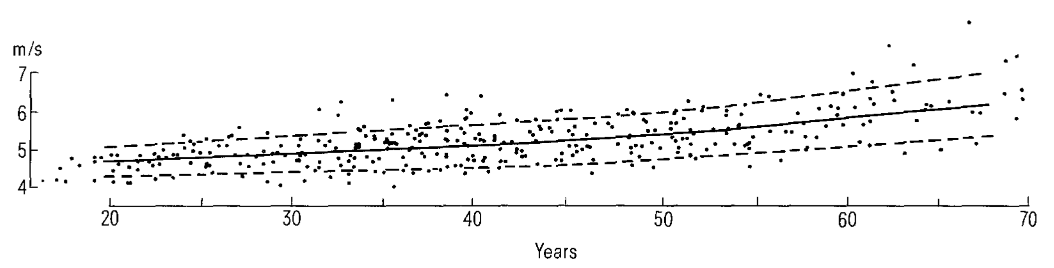

Figure 2. Transversal (cross-sectional) plot of pulse wave velocity (PWV) in m/s against age in years. The mean values (median line) and the limits of standard deviation (broken line) increase with age.

tively fast alteration of the arterial walls (No. 41 in fig. 3A, No. 37 in fig. 3C, etc.).
Individual behavior can also be distinguished by separating smooth from uneven PWV lines. Despite unavoidable variability in conditions of measurement, some PWV lines are surprisingly smooth (No. 108 in fig. 3A, No. 4 in fig. 3B, No. 57 in fig. 3C, etc.). Other PWV lines exhibit ups and downs, which may indicate variations in health status and/or measurement conditions (No. 17 and 41 in fig. 3A, No. 76 in fig. 3B, etc.).

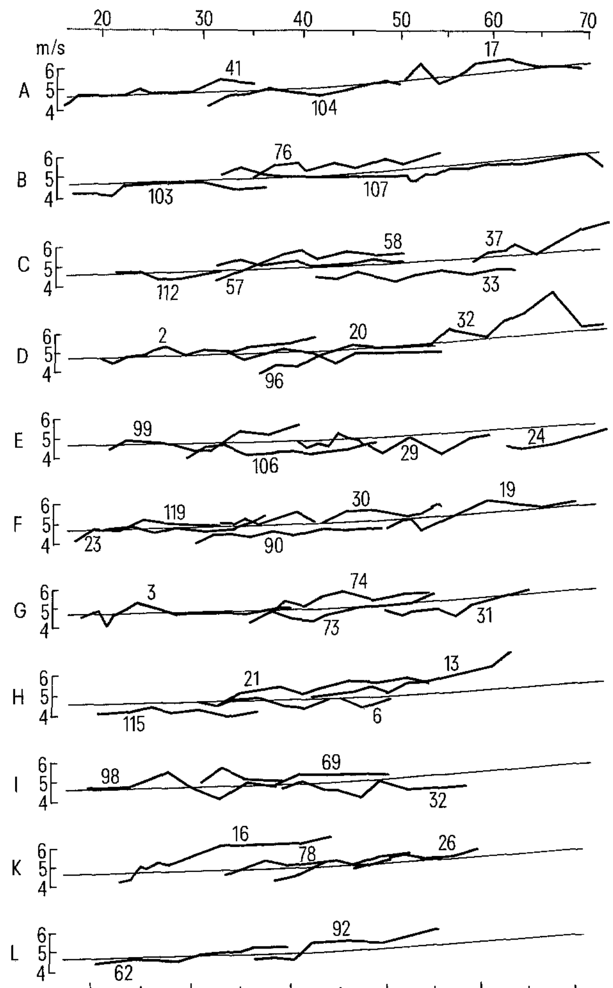

Figure 3. Longitudinal series of measurements in 41 subjects compared to the median line (fig. 2). Pulse wave velocity in m/s plotted against age in years.

For statistical evaluation of PWV trends, the individual PWV lines have been collected into 4 groups of longitudinal lines shown in figure 4 as the 4 lines numbered 1, 2, 3, 4. They correspond to the 4 age classes listed in table 2. Each of these 4 age class lines cross the cross-sectional median at a defined age, and is characterized by an 'onset' value below and an 'offset' value above the cross-sectional median. The increase of PWV with age is confirmed by the increase in the 'onset' and 'offset' values in table 2. Note that the significance of the PWV increase is particularly high in age classes 2 and 3, which contain the largest numbers of individuals.

Discussion

Our 20-year-study confirms the age-dependency of PWV reported by previous investigators[1,3-5]. In cross-sectional analysis the average increase in PWV in a stable population of healthy men between 20 and 30 years of age was +0.16 m/s, and +0.45 m/s in men between 50 and 60 years.

Variability of the method. The average transit time of the pulse wave was calculated from 10 separate beats per proband at the same hour of the morning. Since the probands were not available for the rest of the day, PWV variation within one day and from one day to the next

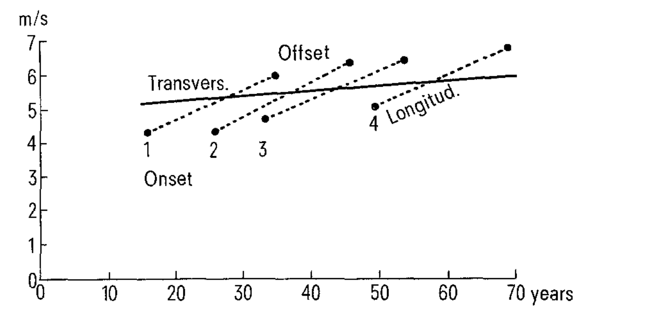

Figure 4. Comparison of longitudinal and transversal data. The 4 longitudinal groups (1, 2, 3, 4) correspond to the classes in table 2. 'Onset' and 'Offset' indicate mean age of probands in the respective class at the beginning and end of the longitudinal study.

Table 2. Numerical and statistical data for the 4 longitudinal classes shown in figure 4.

Age class (years)		Onset	Offset	Difference	Significance
	x̄	4.27	6.03	+1.76	Z = −2.52
6–19	SD	0.4	0.6		*
Class 1	N	10	10		
	x̄	4.40	6.39	+1.98	Z = −3.29
20–29	SD	0.3	0.4		***
Class 2	N	17	17		
	x̄	4.73	6.36	+1.63	Z = −4.78
30–39	SD	0.4	0.5		***
Class 3	N	33	33		
	x̄	5.08	6.75	+1.66	Z = −2.023
40–61	SD	0.3	0.8		*
Class 4	N	7	7		

could not be determined. The 20% variation found in several individuals at the yearly intervals (fig. 3) could, however, closely represent daily variability of the method.

PWV, physiological age, and life expectancy. Knowing from the cross-sectional study that PWV increases with age, it was tempting to postulate that PWV values could allow an estimation of the 'physiological' age of an individual. However, our results were too complex to allow such a simple interpretation. Not all individual PWV lines follow the average trend, as expressed by the median line. In many of our subjects we found quite different behavior: in some individuals, PWV remained constant over a long period of time, only to increase abruptly at an advanced age. In others, PWV increased steadily but much faster than the average trend. This great variety in PWV behavior among individuals even in such a comparatively homogeneous group of probands suggests that PWV in itself is not sufficient to determine the 'physiological age' of an individual. For the same reason, we have no data which would support a strong correlation between PWV values and life expectancy.

Increased PWV and mean arterial blood pressure. Our longitudinal study of individual behavior reveals a trend towards an increased PWV in most individuals over the years. Continuous high PWV in probands No. 58 and 76, and a late rapid increase in probands No. 37 and 41 are exceptions, possibly to be accounted for by an increased mean arterial blood pressure. The high mean PWV shown in figure 3 above 50 years is strongly determined by a few unusual individuals (i.e., No. 37 and 38).

In order to understand why these few probands exhibited an unusual increase in PWV under conditions other than physiological age (fig. 3, No. 37, 38, 41, 58, 76), we compared their individual behavior with their mean arterial blood pressure, measured by O. Gsell[6] on the same probands of our Basel study group. We used 3 of the 4 longitudinal average curves for PWV shown in figure 3 and the statistical data in table 2. As for PWV we calculated 'onset' and 'offset' values of mean blood pressure (in mm Hg) for each of the 3 longitudinal curves. These blood pressure data can be summarized as follows: Class 2 (20–29 years, 17 probands): onset 91.7 ± 5.4 mm Hg, offset 103.1 ± 10.6 mm Hg. Difference +11.4, high significance (Z = −3.5); Class 3 (30–39 years, 33 probands): onset 93.9 ± 8.1 mm Hg, offset 101.5 ± 0.7 mm Hg. Difference +7.6, high significance (Z = −3.8). In Class 4, with only 7 probands between 40 and 61 years, the onset/offset values are considerably higher (105.7 and 116.0 mm Hg), with a difference of +10.3 mm Hg, but in view of the small number of probands, significance is low.

Comparison between PWV and mean blood pressure in the 3 classes suggests that Class 4 (40–61 years) contains the 'continuously high' or 'late fast increase' probands (among them a few cases of hypertonia) and, above 50 years, the high average values of the 'unusual' probands, and that both PWV and mean blood pressure follow similar age patterns.

Extensibility, elasticity and biochemical alteration of the arterial wall. Moret[5] measured arterial pressure and PWV between the carotid and the popliteal arteries in 51 subjects from 13 to 80 years; this arterial system included the thoracic aorta. Moret calculated the extensibility module in vivo according to the formula of Bramwell, Dowling and Hill[8]:

$$PWV = K\frac{dp}{dv}V \text{ (coefficient K = 0.357)}$$

where $\frac{dp}{dv}$ is the extensibility, and $\frac{dp}{dv}V$ the extensibility module. For comparison, he also calculated the extensibility module in vitro of aortic segments for an arterial pressure of 100 mm Hg. The extensibility value for the thoracic aorta is mainly conditioned by the elastic fibers. It would be erroneous, however, to support Moret's assumption that the elasticity of the abdominal aorta is the same as that of the thoracic aorta. The comparison showed that the extensibility curves exhibited similar slopes, demonstrating an increase of PWV and loss of elasticity of the arterial system with increasing age. The in vivo values are lower than the in vitro measurements. The extensibility of the aorta increases slightly until the age of 60, at a physiological mean arterial pressure of 100 mm Hg. In contrast, the extensibility module increases already from age 20 onwards if the mean pressure is high (150 mm Hg). As far as the thoracic aorta is concerned, the change in extensibility is mainly due to alterations in both collagenous and ground substances. Elastic fibers keep their properties until their distensibility becomes altered around age 70.

In our study, individuals with a constantly high PWV or a late abrupt increase were exceptional, as mentioned above. Among factors possibly involved in this increase, we mentioned a connection with increased mean arterial blood pressure. It seems that in these cases factors other than increasing age must be involved, such as alterations of other components of the arterial wall. Only modern biochemical analysis will be able to shed new light on this problem.

1 Monnier, M., Geront. clin. *9* (1967) 81.
2 Boucke, H., and Brecht, K., Dt. med. Wschr. *77* (1952) 562.
3 Wezler, K., and Standl, R., Z. Biol. *97* (1936) 265.
4 Peterson, L.H., Jensen, R.E., and Parnell, J., Circulation *8* (1960) 622.
5 Moret, P.R., Biblthca cardiol. *15* (1964) 40.
6 Gsell, O., Prakt. Geriat. *44* (1973) 284.
7 Bruckner, R., Geront. clin. *9* (1967) 87.
8 Bramwell, J.D., Downing, A.C., and Hill, A.V., Heart *10* (1923) 289.

0014-4754/87/040378-04$1.50 + 0.20/0