

NORMAL HUMAN AGING:
The Baltimore Longitudinal Study of Aging

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NIH Publication No. 84-2450
November, 1984

CHAPTER IV

Tests Administered

INTRODUCTION

The Baltimore Longitudinal Study of Aging (BLSA) is unique among longitudinal studies in the large number of tests that have been administered serially in individuals ranging in age from 17 to 103 years. Observations include medical, genetic, biochemical, physiological, and behavioral variables. The comprehensive nature of the data base makes possible systematic description of the relations among aging trends in various types of human performance that may increase our understanding of the mechanisms of aging.

This chapter describes the tests administered and the procedures followed. Although some of the tests and the conditions under which they were administered have been published, many have not. Some of the tests and procedures are thus described in detail, while others are documented by references to pertinent articles in the literature. Information is provided about methods, the periods when the tests were administered, and the subjects who were tested. Table 1 lists the tests administered, as well as the number of data points for each as a function of number of subject visits. All data reflect our cumulative experience through June 30, 1981.

CLINICAL EVALUATION

1. History

In advance of each visit to the Gerontology Research Center (GRC), the subject is sent a medical-history questionnaire to complete and bring to the Center. During the interview, the examining physician reviews the information with the subject and if necessary supplements it. The history is further supplemented, when indicated, by summaries of hospital or physicians' records.

2. Physical Examination

At each visit a history and physical examination are recorded by staff members of the GRC on standard forms. The physicians, who usually serve two- or three-year National Institutes of Health fellowship appointments, are selected because of their interest in one of the research programs of the GRC. Most spend six to eight hours per month conducting physical examinations of the longitudinal subjects; the rest of their time is devoted to clinical and laboratory research in the operating section of the GRC to which they have been recruited.

When special diagnostic tests are necessary, they are conducted in the clinical facilities of the Baltimore City Hospitals (BCH). All reports of physical examinations are reviewed by a senior staff physician. After review, the clinical and laboratory data are entered in the central data bank and a summary report is sent to the subject's private physician.

Table IV.1. Tests Administered

	Total No. of Tests	Number of Subjects Tested			
		One or More Times	Two or More Times	Three or More Times	Four or More Times
Medical History, Physical Examination, and Laboratory Tests	7395	1142	1006	883	766
Medical history					
Physical examination					
Urinalysis					
Hemogram					
Hematocrit					
Hemoglobin					
Red-cell count					
White-cell count—differential					
Serological test for syphilis					
Chest x-ray					
Resting electrocardiogram	7339	1141	1004	883	762
Cornell Medical Index	2243	1114	649	361	89
Smoking history	7391	1142	1006	882	765
Genetic Factors					
Family history	1142	1142			
Taste test (PTC)	977	977			
Blood typing	766	766			
Dermatoglyphics	682	682			
Lateral dominance	605	461	144		
Biochemical Tests					
Serum albumin and globulin	163	161	2		
Plasma cholesterol and triglycerides	4655	944	812	715	606
Plasma creatinine (see below, "Renal Function")	2049	761	629	438	155
Plasma testosterone	83	83			
Plasma pituitary hormone (AVP)	66	66			
Body Composition					
Anthropometry	7395	1142	1006	883	766
Standing measurements					
Biacromial and waist diameter					
Chest, waist, and buttock depths					
Elbow and neck circumferences					
Height					
Weight					
Skinfold thickness					
Bilateral back, arm, abdomen, and chest					
Chin					
Recumbent measurements	4188	1083	824	668	549

Table IV.1. Tests Administered—(Cont'd.)

	Number of Subjects Tested				
	Total No. of Tests	One or More Times	Two or More Times	Three or More Times	Four or More Times
Body Composition—(Con't.)					
Anthropometry—(Con't.)					
Recumbent measurements	4188	1083	824	668	549
Circumference at iliac crest					
Circumference at greater trochanter					
Length of humerus—right and left					
Circumference of arm at humerus mid-point—right and left					
Length of ulna—right and left					
Circumference of forearm at ulna mid-point—right and left					
Length of femur—right and left					
Circumference of leg of femur mid-point—right and left					
Length of fibula—right and left					
Circumference of calf at maximum— right and left					
Bone density and diameters					
Bone density					
Hand x-rays	2664	1028	697	464	274
Photon scanning	1026	601	317	94	13
Bone diameters from x-rays					
Midshaft overall diameter (hand)	2664	1028	697	464	274
Midshaft medullary diameter (hand)					
Midshaft CCT (hand)					
Metacarpal length (hand)					
Elbow					
Knee					
Wrist					
Ankle					
Body fat thickness from x-rays	377	374	3		
Lean body mass—Behnke Index	4095	1068	797	664	543
Basal metabolism	5672	1092	911	779	636
Creatinine excretion—24-hour	6595	1133	986	816	705
Body water					
Total body water (antipyrine dilution)	1008	717	236	51	4
Extracellular water (thiocyanate dilution)	1078	761	261	52	4
Body density (helium displacement)	132	132			
Nutrition					
Nutrition history	3794	960	791	638	519
Seven-day diet diaries	2755	845	614	489	362

Table IV.1. Tests Administered—(Cont'd.)

	Total No. of Tests	Number of Subjects Tested			
		One or More Times	Two or More Times	Three or More Times	Four or More Times
Neuromuscular function and exercise					
Tapping test	6228	1072	934	810	690
Reaction time					
Touch	1833	782	544	310	148
Simple and choice auditory	1332	675	396	178	61
Reflex time	1317	658	407	177	60
Nerve conduction velocity, motor	2234	834	626	402	237
Exercise screening tests					
Strength test	3697	970	808	603	456
Hand dynamometry—right and left Bilateral adductors, abductors and rotors					
Low-level crank-turning ergometer	3295	924	747	543	408
Maximum work rate—brief	3294	924	747	543	408
Submaximal exercise	630	466	151	13	
Metabolic response—O ₂ & CO ₂ Ventilation					
Blood pressure and pulse rate					
Blood chemistry	200	178	22		
Lactic acid					
Growth hormone					
Activity history questionnaire	5042	1077	865	727	619
Renal Function					
Creatinine clearance—24-hour	4435	1078	875	661	542
Urinalysis (see above, "Medical History, Physical Examination, and Laboratory Tests")					
Pulmonary Function					
Spirometry	6430	1109	945	813	711
Vital capacity—standing and recumbent					
Inspiratory and expiratory reserve volume					
Forced expiratory volume 0.5 and 1.0 sec					
Expiratory flow rates					
Maximum breathing capacity					
Pulmonary gas distribution indices (nitrogen wash-out)	1438	681	477	189	69
Total lung volume					
Residual volume					
Pulmonary and chest compliance	42	42			
Chest x-ray (see above, "Medical History, Physical Examination, and Laboratory Tests")					

Table IV.1. Tests Administered—(Cont'd.)

	Total No. of Tests	Number of Subjects Tested			
		One or More Times	Two or More Times	Three or More Times	Four or More Times
Cardiovascular Function					
Resting electrocardiogram	7339	1141	1004	883	762
Exercise stress electrocardiogram					
Master two-step test	3408	868	687	536	414
Graded treadmill test	2283	771	588	411	262
Systolic time intervals	326	326			
Echocardiography					
One-dimensional	270	258	12		
Two-dimensional	473	473			
Thallium scan of heart (exercise)	356	356			
Gated blood-pool scan	61	61			
Responses to cardiovascular drugs	89	89			
Volume plethysmography	140	140			
His-bundle recording (non-invasive)	80	80			
24-hour ambulatory ECG	150	150			
24-hour ambulatory blood pressure	50	50			
Plasma catecholamines (exercise)	30	30			
Carbohydrate Metabolism					
Oral glucose tolerance test					
1.75 g/kg body wt	1248	871	309	58	9
40.0 g/m ² surface area	842	562	280		
Intravenous glucose tolerance test	1223	767	366	78	11
Cortisone-glucose tolerance test	774	581	160	30	2
Tolbutamide response test	1088	718	305	59	6
Glucose clamp	484	334	108	34	11
Exercise—I-V glucose tolerance	88	88			
Immune System					
Skin Fibroblast Culture					
	434	344	90		
Perception					
Tonography	3489	935	729	588	456
Visual screening test	5721	1038	901	784	664
Acuity					
Color vision					
Visual fields					
Audiometry—pure and pulse tone	4264	1012	845	683	557

Table IV.1. Tests Administered—(Cont'd.)

	Total No. of Tests	Number of Subjects Tested			
		One or More Times	Two or More Times	Three or More Times	Four or More Times
Cognitive Performance					
Learning					
Serial	1785	905	606	274	
Paired associate	1739	891	589	259	
Southern California tests of intellectual abilities	1378	824	554		
Word fluency					
Associational fluency					
Ideational fluency					
Consequences					
Intelligence tests					
Army Alpha	2939	1055	674	395	212
Wechsler Adult Intelligence Scale (Vocabulary only)	2045	1039	645	285	76
Benton Visual Retention Test	2043	1038	644	286	75
Logical problem solving					
I	529	304	225		
II	476	305	171		
Concept problem solving	1300	841	459		
Memory and response time	334	334			
Immediate free recall					
Delayed memory (recall and recognition)					
Digit memory					
Dichotic listening					
Simple and complex decision time and accuracy					
Personality, Behaviors, and Traits					
Guilford-Zimmerman Temperament Survey	2171	1075	681	327	83
Burgess-Caven-Havighurst attitude questionnaire	2122	1100	608	313	80
Questionnaire on attitudes toward old people	1243	811	360	67	5
Personal interview—family and sex history	777	777			
Imaginal Processes Inventory	613	613			
Thematic Apperception Test	66	66			
Stress-and-coping interview	116	116			
Schedule of life events	394	394			
Eysenck Personality Inventory	394	394			
Daily events check list	401	401			
Well-being assessment sheet	407	407			
Profile of mood states	394	394			
Parent-child relations questionnaire	337	337			
NEO Inventory	348	348			
Defense-mechanism inventory	140	140			
Social desirability scale	275	275			
Coping					
Self-Interview	76	76			
Questionnaire	149	149			
NEO Rating Inventory	146	146			

3. Laboratory Tests

Oral/dental examination. Since 1978, the oral health status of approximately one third of the BLSA subjects has been evaluated (Baum, 1981a). Data collected include the number of decayed (coronal, cervical), missing, and filled teeth, and indices of gingival and periodontal disease (Ramfjord, 1967). Oral mucosal tissues are examined. Two dental bite-wing and one panoramic x-rays are taken. A detailed history of oral-hygiene habits, dental treatment, subjective complaints, and nutrition is obtained. Oral motor function is evaluated by physical examination (Bosma, 1976; Baum and Bodner, 1983). Assessments of swallowing, tongue and labial postural function, and masticatory ability are included.

Gustatory function is estimated in two ways: a) by determination of detection thresholds for four taste qualities, sweet (sucrose), salty (sodium chloride), sour (citric acid), and bitter (quinine sulfate) (Weiffenbach et al., 1982); and b) by determination of suprathreshold taste intensity through a magnitude-estimation procedure for each testant (Bartoshuk, 1978; Cowart and Baum, 1981). Salivary-gland function is assessed by collecting unstimulated whole saliva (volume determination after expectoration) and 2% citric acid stimulated parotid saliva (Baum, 1981b). From parotid saliva collections the following information is routinely obtained: flow rate, protein by A_{215} , K^+ , Na^+ , Ca^{++} by atomic absorption spectrometry; inorganic phosphate (Chen et al., 1956); anionic proline-rich proteins and amylase (by immunochemical assays).

Urinalysis. Routine clinical urinalysis, including microscopic examination of urinary sediments, is performed at each visit.

Hemogram. Hemoglobin, hematocrit, white-cell, and differential counts are made on each visit.

Serological test for syphilis is made on the first and every subsequent fourth visit.

Total serum protein, albumin, and globulin. These analyses were made at each visit from 1958 to 1969 in venous-blood samples drawn after 12 hours' fasting (7:00 P.M. to 7:00 A.M.).

Cholesterol. All determinations are made on fasting blood samples. Serum concentrations of cholesterol have been measured at each visit since April 1962 by the method of Abell et al. (1952) as modified by Anderson and Keys (1956). The method of Blankenhorn et al. (1961) has been used in the initial serum preparation phase so that triglycerides and cholesterol can be assayed on the same serum specimen. Since April 1969 all sera have been assayed by BioScience Laboratories, Van Nuys, California; in addition, once each month one eighth of the monthly samples are assayed in the GRC laboratory so that the BLSA can maintain its own reference method as a continuing quality control. From these analyses conversion factors have been developed for the BioScience analyses (Hershcovf et al., 1982). The BioScience Laboratories utilized the analytical method of Kessler (1967) until July 1970. Since that time the method of Wybenga et al. (1970) has been used.

Triglycerides. Serum concentrations of triglycerides have been measured at each visit on fasting blood samples by the method of Blankenhorn et al. (1961). Analyses by BioScience Laboratories use the method of Kessler (1967). Monitoring by BLSA is the same as for cholesterol (see above).

4. The Cornell Medical Index Health Questionnaire

The Cornell Medical Index Health Questionnaire (Brodman et al., 1949, 1960) is

administered on visits 1, 5, 9, etc. The subject responds to 195 questions about his symptoms, health habits, and family history.

GENETIC CHARACTERISTICS

1. Family History

A detailed family history is obtained on the first visit, entered in the central data file, and updated on each subsequent visit.

2. Genetic Markers

Since the following tests concern characteristics that are assumed not to change with age, they are administered only once, usually but not always at the first visit.

Taste Test. The ability to taste phenylthiocarbamate (PTC) is recorded during the first visit. PTC is administered on filter-paper strips placed on the subject's tongue.

Blood Types. Typing for ABO, MN, Rh, Kells, Kidd, and Duffy factors is carried out at first visit only.

Dermatoglyphics of the Hand. Finger and palm prints of all subjects are collected by the Faurot inkless method with the digits comfortably extended during printing (Plato, 1978). The prints are classified according to the methods of Cummins and Midlo (1943).

Handedness. Hand, foot, and eye preference are determined to infer hemispheric dominance. Single-handed, two-handed, foot, and eye function are determined by a series of standard tasks.

BODY STRUCTURE AND COMPOSITION

1. Anthropometry

As many as 37 anthropometric measurements are made on all subjects in the standing and recumbent positions at each visit. Standard techniques use tapes for circumferences and lengths, calipers for diameters. In addition to height and weight measured without clothes, eight diameters, 12 circumferences, and eight lengths are measured. Skinfold thickness is measured with a constant-pressure Harpenden caliper at nine standard sites (Edwards et al., 1955; Tanner, 1955).

2. Bone-Mineral Measurements

Bone density and diameters from hand x-rays. Radiographs of each hand are taken postero-anteriorly at an average exposure of 1.0 sec at 100 mA and 60 kVp without intensifying screens. All measurements are made on the second metacarpal bones as described by Garn (1954, 1961, 1970). Specifically, the total width, medullary width at the midshaft of the bone, and length along the longitudinal axis are measured.

Bone density from photon scan. The Cameron technique (Cameron and Sorenson, 1963, 1968) of bone-mineral analysis is used to determine the mineralization of the radius and the ulna in both arms. This technique passes a collimated beam of monoenergetic photons through the combination of soft tissue and bone in a limb, and the resulting attenuation is monitored with a suitable photon detector. The source and

detector are moved across the limb; the resulting absorption curve can be related directly to the scan. The Norland-Cameron Analyzer, an automated instrument used since 1972 for the determination *in vivo* of bone-mineral content, provides direct digital readouts of both bone-mineral density and bone width without external calculation or manipulation of data.

3. Subcutaneous Fat

In addition to measurements of skinfold thickness, estimates of subcutaneous fat were made from soft-tissue radiographs. From 1958 to 1973, measurements were made at each visit. Each 7-x-17-inch film contains views of seven sites on the trunk and limbs. Radiographic procedures and techniques were those described and illustrated by Garn (1954, 1961).

Measurements of skin and fat combined were made by means of a Helios dial-reading caliper calibrated to 0.05 mm (Borkan and Norris, 1977). Sites of fat measurements on the trunk were bony landmarks, such as the top of the greater trochanter. For measurements on the calf and forearm, the widest part of the limb was chosen. Fat measurements were made at the following locations on each film: anterior calf, posterior calf, medial calf, lateral calf, lateral to greater trochanter, lateral to top of greater trochanter, lateral to anterior-superior spine of iliac crest, lateral to top of iliac crest, lower part of thorax (lowest rib), medial arm, and lateral arm. The average value for reliability coefficients determined by duplicate measurements on 20 films was 0.95 (Borkan and Norris, 1977).

4. Estimates of Components of Body Mass

Behnke index. A quantitative classification of body build devised by Behnke is based on 11 circumferences and eight diameters of the body. Factors are used to indicate numerically the degree of fatness, muscularity, and skeletal size (Behnke, 1961, 1963). Measurements were made every fifth visit from 1961 until 1972; since then, measurements have been made at each visit.

Muscle mass. The total amount of creatinine excreted in 24 hours, measured at each visit, is used as an index of muscle mass (see below, "Renal Function").

Body-water compartments—estimates of muscle and fat. The volume of distribution of antipyrine is used to estimate total body water (Soberman et al., 1949). Thiocyanate space is used as the estimate of the volume of extracellular water (Levitt and Gaudino, 1950). Subjects who report a history of significant allergic reactions are not tested. Subjects tested between 1958 and 1967 received an intravenous infusion of antipyrine (1.0 g) and sodium thiocyanate (1.396 g) in 50 ml of normal saline. Plasma levels of antipyrine and thiocyanate were measured in peripheral venous blood at 2, 4, 6, and 8 hours after the infusion began. Antipyrine was estimated by the method of Brodie and associates (1949), with modifications (Shock et al., 1963). Thiocyanate levels are estimated by the method of Bowler (1944). Volumes were estimated from the slopes of the linear regressions of the logarithm of the concentrations of antipyrine and thiocyanate in the four blood samples. Intracellular water, calculated as the difference between antipyrine (total body-water volume) and thiocyanate (extracellular water volume), was used as an index of fat-free tissue.

Body density—body fat. From 1959 to 1966, estimates of body density were made by the Siri technique (Siri, 1956), by which the subject sits in an airtight chamber into which a known amount of helium is introduced. The temperature and helium

concentration in the air surrounding the subject were monitored continuously for 15 minutes, by which time equilibrium is achieved. The body volume of the subject was calculated from the helium concentration in the air surrounding the subject. Body density was obtained from the ratio of total body weight to body volume. With appropriate corrections for bone density, an estimate of body fat was made for each subject. Quality control was maintained by the introduction of standard carboys of known volume into the chamber for measurement each day (Norris et al., 1963; Siri, 1962).

Because of excessive operating costs and technical difficulties, this measurement was discontinued in 1966.

5. Strength Tests (see below, "Neuromuscular Function and Exercise")

6. Basal Metabolism

Basal oxygen uptake is determined at each visit by the open-circuit method described by Shock and Yiengst (1955). Three ten-minute samples of expired air are collected on each of two consecutive mornings after the subject's overnight stay at the GRC. Until 1965, aliquots of expired air were analyzed for O₂ and CO₂ content by the Haldane apparatus. Between 1965 and 1968, expired air was also analyzed for O₂ by the paramagnetic method (Beckman Paramagnetic O₂ Analyzer, Model G-2), and for CO₂ by infrared absorption (Beckman Model LB-1). Once the two analytical systems were shown to be equivalent, all subsequent analyses were done by the more modern methods. Both instruments are calibrated daily with standardized mixtures of O₂ and CO₂ obtained commercially in standard pressure tanks and checked by the Haldane method (Tzankoff and Norris, 1977).

This test also provides data on basal respiratory volume, CO₂ elimination, heart rate, and blood pressure. Blood pressure and heart rate are recorded by nursing personnel.

NUTRITION

1. Dietary Habits

From 1961 to 1965, and from 1968 to 1975, assessment was made of dietary habits of the subjects. On the first day of a visit, after a brief introductory review of his diet, each subject was instructed by a trained nutritionist in the keeping of an accurate dietary diary. Various plastic food models were used to teach subjects appropriate assessment of portion size. For practice, a trial diary kept during the subject's visit at the GRC was monitored by the nutritionist. A seven-day food record, begun on the subject's return home, was mailed to the nutritionist. From 1961 to 1965, a subset of the subjects collected seven-day records at three-month intervals. All records were verified by the nutritionist with the subject on his next visit to the GRC.

Data from each record were coded by the nutritionist and entered on tabulation cards for computer analysis of mean daily nutrient intakes, variances, and the major food-group sources of the nutrients by a computer program developed by the Heart Disease Control Program, Bureau of State Services, United States Public Health Service. The analysis identified average daily intakes of total calories, protein, total fat, saturated fatty acids, oleic acid, linoleic acid, linolenic acid, and other fatty acids, total

carbohydrates, simple and complex carbohydrates, alcohol, cholesterol, fiber, calcium, iron, and selected vitamins (vitamin A, thiamine, riboflavin, niacin, pyridoxine, and ascorbic acid). In addition, certain derived variables were computed (percentage of calories from protein, carbohydrate, and fat, and the polyunsaturated/saturated fatty-acid ratio) (McGandy et al., 1966).

2. Nutrition History

A nutrition history was recorded in a personal interview by a trained nutritionist during the periods 1961–1965 and 1968–1975, in order to detect unusual dietary features that might not have been revealed by the dietary diary.

3. Pyridoxine (Vitamin-B₆) Status

These studies were carried out from 1971 to 1973 (Rose et al., 1976). Venous-blood samples were obtained one to two hours after breakfast. Vitamin-B₆ supplementation by intake of vitamin pills was established from the patient's history. Plasma pyridoxal phosphate (PLP) was determined by a modification of the method of Chabner and Livingston (1970). Erythrocyte glutamic-oxaloacetic transaminase (EGOT) activity was determined by AutoAnalyzer Method No. 3 (Technicon Instruments Corp., Tarrytown, New York). EGOT was also measured after *in-vitro* stimulation with pyridoxal phosphate.

NEUROMOTOR FUNCTION AND EXERCISE

1. Tapping Test

This test, designed (Welford et al., 1969) to evaluate speed and accuracy in a visually controlled motor task, records the time required to place pencil dots alternately on two targets drawn on paper. A number of separate tests of increasing difficulty are carried out at each session. In these trials subjects are instructed to complete the task as rapidly and accurately as possible. Three sets of targets are used. Each set consists of pairs of vertical lines requiring movements of 50, 142, and 402 mm. The target widths (distances between the two parallel lines) are 32, 11, and 4 mm. The nine combinations of movement distance and target width are presented in different orders to different subjects in such a way that the serial positions both of the conditions and of the transitions from any condition to any other are appropriately balanced. Subjects have one trial with each of the conditions for practice, followed by a second trial with each pair of targets; their scores are derived from the second. The test was administered on each visit from 1960 to 1981.

2. Oral Motor Evaluation (see above, "Oral/dental examination")

3. Reaction Time

Reaction time to touch. Foot-reaction times are measured after stimulation by touching the sole of the foot. The subject is instructed to plantar-flex his foot as soon as possible after the touch. Tests are performed with the subject in the dorsal recumbent position. The muscle-action potentials are led from small solder electrodes placed over the small muscles of the left foot through paired leads and a preamplifier to a dual-beam cathode-ray oscilloscope. The subjects are connected with ground through a

saline-moistened pad placed around the leg just above the ankle. The action potentials are recorded photographically from the oscilloscope screen.

Tactile stimuli to elicit voluntary muscle reactions are provided by a plastic rod tapered to a dull point at one end. Contacts are placed in the handle of the rod so that at the instant of contact with the skin the beam of the cathode-ray oscilloscope is triggered. The reaction times are measured by the interval between the beginning of the cathode-ray beam and the beginning of the muscle-action potential. A time-calibration curve (10 msec) is recorded on the second synchronized beam from the output of a standard oscillator. For each subject, mean values for reaction time are calculated after exclusion of the extreme high and low values, defined as those separated from the remainder of the distribution by an interval of more than 15 msec (Hügin et al., 1960).

This test was administered on alternate visits from 1958 to 1974. Since 1974, it has been administered on each visit.

Auditory reaction time. Between 1961 and 1976, simple and choice reaction times were measured concurrently with electroencephalographic (EEG) recordings from the occipital region of the head (Surwillo, 1963a). The stimuli were tones (250 Hz and 1000 Hz) presented over a loudspeaker at a level set by each subject to ensure that they were clearly audible. The duration of each stimulus was 0.3 second. No forewarning was given of the stimuli, which were triggered by the experimenter when alpha waves were evident on the EEG. In simple reaction time, the task was to press a button on a hand-held switch as quickly as possible whenever either tone was presented. In choice reaction time, the task was to press the button only when the higher tone was presented.

In 1974, a study was initiated in which only simple reaction time is measured; both tones are used. EEGs are no longer recorded, and the stimuli are triggered by the experimenter at random intervals of 10 to 25 seconds.

Reflex time. Reflex times are measured for stimuli applied to the sole of the foot. Plantar flexor reflexes are elicited by a brief longitudinal scratch applied with a plastic rod to the sole of the foot in the area in which the shortest, most consistent responses are obtained. The length of this scratch is approximately 5 cm, the duration approximately 200 msec. The intensity of scratch necessary to elicit flexion potentials ranges from a mere touch to a firm stroke. In no case has the stimulus been judged painful by the subjects.

A dual-beam oscilloscope is used to record the application of the stimulus and the muscle-action potentials (see above, "Reaction time to touch").

Fifty or more stimuli of each type are administered to each subject at each sitting. Average values are calculated if nine single values are obtained from the records (Hügin et al., 1960; Magladery et al., 1958).

Plantar reflex times were recorded in all subjects on alternate visits from 1958 until 1974, and have been recorded on each visit since 1974.

4. Nerve-Conduction Velocity

Nerve-conduction velocity is estimated from the time elapsing between the percutaneous application of an electrical stimulus to the ulnar nerve and the motor response in the muscles of the hypothenar eminence and the linear distance traversed. Electrical stimuli (supramaximal square wave shocks of 0.1 msec duration) are applied

to the left arm over three separate points of the ulnar nerve, at the wrist, at the elbow, and 5 cm below the axilla. The cathode of the stimulating electrode is always placed over the part being stimulated. The response, recorded on a cathode-ray oscilloscope, is the action potential evoked in the muscles of the hypothenar eminence by the electrical stimulus to the nerve. One recording electrode is placed over the bellies of these muscles and the other over the tendons. Latency of response (the time between stimulation and the onset of the action potential of the muscles) is measured to the nearest 0.1 msec. Distances along the skin surface between centers of the stimulating electrodes and the recording electrode are measured to the nearest 0.1 cm (Norris et al., 1953; Wagman and Lesse, 1952).

This test was administered on alternate visits between 1958 and 1974.

5. Physical Activity: Muscular Strength and Work

Physical-activity history. The average level of physical activity was initially estimated from a personal interview or questionnaire (McGandy et al., 1966), and latterly by the questionnaire, which covers specific activities at home, at work, and in recreation, as well as variations in activity patterns such as trips and seasonal sports. The amount of time spent in each activity is expressed as a daily average. Time assignable to seasonal activities and activities that are pursued infrequently is expressed as an annual total and divided by 365 to obtain the daily average. The total daily energy expenditures are calculated for each subject by use of the caloric values for each activity determined by McGandy et al. (1966).

Strength tests. Maximum grip strength of right and left hands is estimated with the Smedley hand dynamometer (Fig. 1). All subjects are tested at each visit.

A special instrument was designed and constructed to measure strength of adductors, abductors, dorsal rotators, and ventral rotators of the arms. With this apparatus, the subject is seated with a one-inch bar in front of him. The subject pushes, pulls, lifts, or depresses the bar, which does not move. The force exerted on the bar is recorded by strain gauges. The maximum force developed in the highest of three trials at each maneuver is used as the strength index. The test has been administered to all subjects on each visit since 1960.

Coordinated exercise—cranking. The subject lies on his back on a bed and turns an electrodynamic-brake ergometer crank (Fig. 2) with his arms to accomplish 500 kg m of work at the slow and easy rate of 135 kg m/min (Kelso and Hellebrandt, 1934; Tuttle and Wendler, 1945). The output of the ergometer, which measures the work rate continuously, is monitored for variability of performance of the assigned rate (Norris and Shock, 1957).

Maximum work output is also determined. In this test the subject turns the ergometer crank at his greatest effort (maximum rate) for ten seconds. Four different resistances are used. Maximum work rate achieved at each work load is recorded. The test has been administered to selected subjects at irregular intervals.

6. Physical Activity: Oxygen Uptake

Submaximal workloads. Submaximal work (500 kg m at 135 kg m/min) is performed with the arms on the electrodynamic-brake ergometer (Fig. 2). The subject remains in a recumbent position throughout the periods of rest, exercise, and recovery.

Expired air is collected through a Siebe-Gorman face mask in serial ten-liter



Figure IV.1. Measurement of hand-grip strength by Smedley dynamometry.

samples for 20 minutes before the exercise, during exercise, and for 40 minutes after exercise. The concentrations of O_2 and CO_2 in each ten-liter sample of expired air are determined by automatic gas analyzers (see above, "Body Structure and Composition," "Basal Metabolism"). All gas volumes are reduced to standard conditions ($0^\circ C$, dry, and 760 mm Hg) (Carpenter, 1939). Pulmonary ventilation volume is determined by dividing the volume of air expired during the collection period by the duration of the period.

Rates of O_2 uptake and CO_2 elimination are estimated from ventilation rate and gas concentrations of the expired air by standard metabolism techniques (Peters and

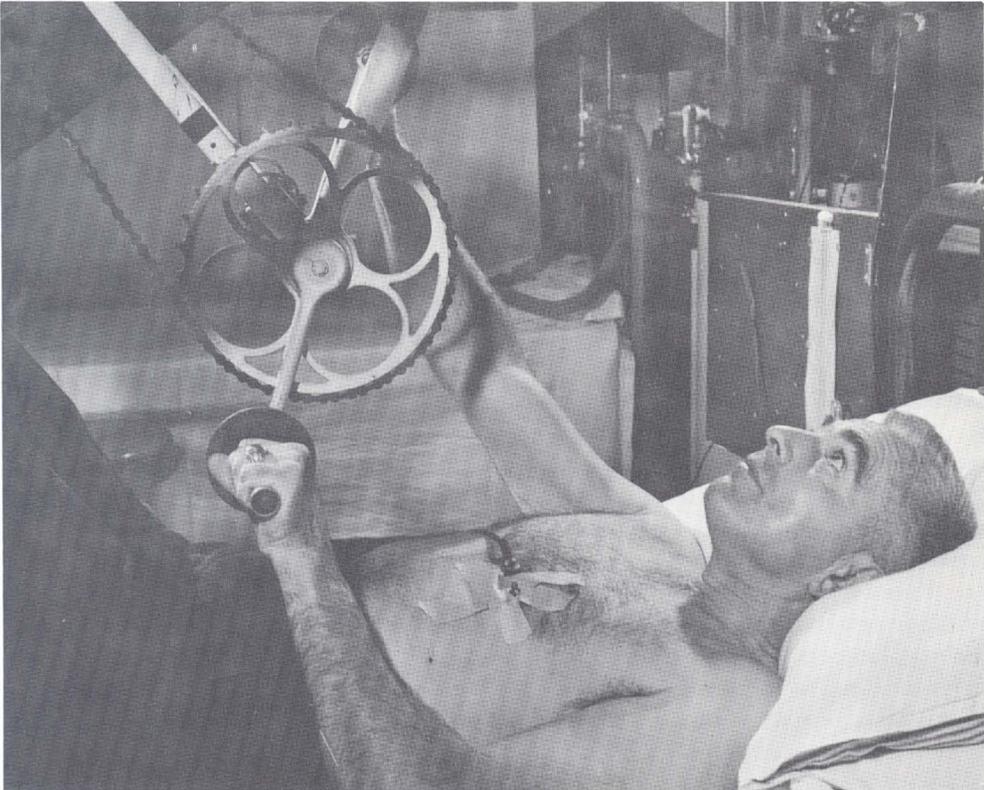


Figure IV.2. Measurement of maximum work by bicycle ergometry.

Van Slyke, 1932). Maximum O_2 uptake, CO_2 elimination, and ventilation volume (l/min) are recorded from the highest value found for the volume in a single period during or after exercise. Total excess values for ventilation, CO_2 elimination, and O_2 absorption are obtained by summing over all periods during and following exercise the amount by which each individual metabolism period exceeds the value of the asymptote of the appropriate recovery curve. Net mechanical efficiency is calculated as the caloric equivalent of the work done, divided by the caloric equivalent of the metabolic cost of the work (total O_2 uptake, during and after exercise). The respiratory quotient is assumed to be 1.0. Oxygen debts are calculated as the amount of excess O_2 absorbed after the end of the exercise (Norris and Shock, 1957).

Measurements of blood pressure and heart rate are also made during the resting period and at 30-second intervals following exercise.

These tests have been applied to selected subjects at irregular intervals.

Maximum oxygen uptake. From 1975 to 1981, O_2 uptake was measured during graded maximal treadmill exercise in participants without clinical or stress electrocardiographic evidence of heart disease. According to the modified Balke exercise protocol (Balke and Ware, 1959), the individual walks at a constant speed throughout the test; the treadmill is elevated 3% every two minutes until the subject is unable to continue because of general fatigue, chest pain, shortness of breath, or leg discomfort. During exercise, the subject breathes through a mouthpiece connected to two large collection tanks by a flexible plastic hose. Expired air is collected for the second minute

of each two-minute stage and analyzed on-line for O₂ and CO₂ content by a Beckman Medical Gas Analyzer LB-2. Correction of prevailing room temperature and barometric pressure permits calculation of the O₂ consumption in ml O₂ per min per kg body weight for each stage of exercise. The value at maximal exercise is generally considered the best available measure of physical fitness.

RENAL FUNCTION

1. Creatinine Clearance (24-Hour)

A non-fasting serum sample for the determination of creatinine levels is obtained from each subject on his arrival at the GRC, between 9:00 and 11:00 A.M. After the subject empties his bladder, a 24-hour urine collection is begun. A fasting blood sample for the determination of serum creatinine is obtained at 8:00 A.M. the next day; the mean of the two determinations is used in the calculation of creatinine clearance. Creatinine in serum and urine is measured as true creatinine, by a modification of the technique of Hare (1950). Clearance values are expressed as ml per 1.73 m² surface area (Rowe et al., 1976a).

In order to establish the validity of creatinine clearance as a measure of glomerular filtration rate, simultaneous inulin and creatinine clearances were performed in healthy male volunteers who were not participants in the BLSA. Creatinine and inulin determinations were made on the same blood samples drawn during a 45-minute intravenous infusion of insulin (Davies and Shock, 1950). The mean ratio of creatinine to inulin clearance was 1.29. Age had no significant effect on the ratio.

Creatinine clearance was determined on all visits from 1958 to 1974. Since July 1974 the test has been administered on visits 1, 2, and 5, and at every fourth visit thereafter.

2. Urinalysis (see above, "Clinical Evaluation")

3. Concentrating Ability of the Kidney

Water-deprivation tests were conducted from 1958 to 1962 (Rowe et al., 1976b). There was no oral intake from 6:00 P.M. to 6:00 A.M.; after the subject emptied his bladder at 6:00 P.M., urine samples were obtained at 9:00 P.M., 12:00 midnight, and 6:00 A.M. for measurement of urine flow and osmolality. At 6:00 A.M. a plasma sample for osmolality and creatinine concentration was obtained. From these data solute excretion and osmolar clearances were calculated. This test was not repeated.

PULMONARY FUNCTION

All pulmonary-function tests were performed at various times of the day.

1. Spirometry

Earlier studies (1958-1962) of vital capacity and pulmonary subdivisions were performed with subjects both standing and recumbent; they inspired maximally and then expired maximally into a 120-liter recording Tissot spirometer. Complete spirograms were also recorded for recumbent subjects with a ten-liter closed-circuit spirometer. A mouthpiece and nose-clip were used for these collections. Three trials

were made for each effort (inspiratory reserve volume, expiratory reserve volume, and vital capacity). The largest value was recorded as the value for the day. The resting tidal volume was determined by dividing the total volume expired in a ten-minute air-collection period by the number of breaths during this period as counted from a kymograph tracing (Norris et al., 1956). Since 1963, spirometric studies have followed methods and calculations described by Kory et al. (1961), including forced expiratory volumes (FEV_{0.5} through FEV_{6.0}). These tests are performed at each visit.

2. Maximum Breathing Capacity

The maximum breathing capacity is determined in standing subjects who are asked to breathe as much air as possible into a spirometer through a low-resistance circuit for 15 seconds. Neither the rate nor the depth of breathing is specified, but the subject is urged to do his best throughout the test. The highest volume attained in three trials is taken as the value for the day (Norris et al., 1956). This test is administered on each visit.

3. Pulmonary Gas Distribution

Total lung volume and functional residual volume were determined by the nitrogen wash-out method using an open-circuit technique (Edelman et al., 1968). The studies were performed at various times of the day with subjects in the seated position. The gas supply and collection bags were enclosed in an airtight box. Tidal volume was monitored by a model 350 Servo-spirometer (Med-Science Electronics, St. Louis) connected to the box. Nitrogen concentration of gas sampled at the mouthpiece was measured with a model 300 AR Nitralyzer (Med-Science Electronics). The instrument was calibrated with five standard gas mixtures within the 2%–7% nitrogen range before each wash-out test. Continuous recordings of N₂ concentration and tidal volume were made with a model 1508 Visicorder (Minneapolis Honeywell Regulator Co., Denver). Subjects were allowed to accommodate to the apparatus while breathing air. A vital-capacity maneuver consisting of a full inspiration followed by a full expiration was performed during this period. After the subjects had returned to a steady ventilatory pattern (usually within 0.5–1.0 min), and at the end of a normal expiration, a seven-minute period of oxygen breathing was begun. Functional residual capacity (FRC) was calculated from the collected expired air. Corrections were made for inspired nitrogen concentration and tissue nitrogen excretion (Darling et al., 1940). Tidal volume was taken as the mean for the seven-minute period; anatomic dead space was estimated from the height of each subject (Hart et al., 1963).

Uniformity of ventilation was initially assessed by the use of the lung-clearance index (LCI) (Becklake, 1952), which was later supplanted by a new index less dependent on tidal volume (Edelman et al., 1968).

This test was administered between 1962 and 1979 to randomly selected subjects.

4. Lung and Chest-Wall Compliance

Since this test required the placement of an intra-esophageal balloon catheter to measure pressures, it was administered to only 42 subjects, aged 24 to 78 years, during 1962 and 1963 (Mittman et al., 1965). Although analysis of cross-sectional data showed a significant negative regression of chest-wall compliance on age, the regression of pulmonary compliance on age was not significant. In view of the discomfort to the subject, the large investment of time required to perform the test, and

the lack of a significant age regression for pulmonary compliance, the test was not repeated after 1963.

5. Smoking History

A detailed smoking history is obtained on the first visit and is updated at each subsequent visit.

6. Chest X-Ray

A standard roentgenogram is made for postero-anterior (P-A) and lateral views of the chest with the subject standing. The total equivalent radiation exposure received by the subject above the waist is 2 rads for the two tests. Gonadal exposure is minimized by standard techniques of collimation and shielding. The techniques and equipment used are monitored by the Maryland State Health Department and the Baltimore City Hospitals' Radiation Safety Officer. X-rays were initially repeated at each visit, but since 1979 have been routinely repeated no more frequently than every five years unless there is a specific clinical indication that more frequent examination is desirable.

CARDIOVASCULAR FUNCTION

1. Screening for Cardiovascular Disease

Cardiovascular history and physical examination. At each visit, in addition to the general history and physical examination, a cardiovascular history and examination are performed by a physician of the GRC Cardiovascular Section, and a 12-lead resting electrocardiogram (ECG) is obtained. Participants who do not demonstrate definite evidence of coronary artery disease (CAD) in this examination undergo exercise stress to detect the presence of occult CAD.

On the basis of the history, physical examination, and stress testing (see below), each participant is classified as showing a) no evidence of CAD or b) evidence of probable or c) definite CAD. The following point system is employed to determine the classification of each individual:

ECG	Points
Resting	
Minn. Code 1:1	2
Minn. Code 1:2	1
Minn. Code 1:3	1
Minn. Code 4:1	1
Stress	
Minn. Code 11:1	1
<i>Angina pectoris</i>	
Possible	0
Probable	1
Definite	2
<i>Previous myocardial infarction (MI)</i>	
Possible	0
Probable	1
Definite	2



Figure IV.3. Treadmill stress electrocardiography.

The presence of angina and/or the history of MI is assessed by a staff cardiologist. Confirmation of prior MI also requires unequivocal hospital records. Assignment to the category of definite CAD requires at least 2 points; to probable CAD, 1 point; and to CAD-free, 0 points. The weight to be attached to the stress thallium scan in classifying CAD is presently under consideration.

Exercise stress test—double Master test. The double Master test was administered to all subjects, except those in whom a clinical contraindication was found, on all visits between 1958 and 1968. The subject climbed two nine-inch steps (total lift = 18 in or 0.46 m) so arranged that at a rate determined by his age and weight he went up two

steps, went down two, turned, and went back the other way. The ECG was monitored (Master and Oppenheimer, 1929; Master et al., 1944).

Treadmill stress ECG. In 1968, a treadmill exercise test (Fig. 3), in which the subject walks on a motor-driven treadmill at a rate of 3.5 miles per hour, was introduced to replace the double Master test. At the end of each two minutes of walking, the grade of the treadmill is increased by 3% (Balke and Ware, 1959). The increments in grade are continued until excessive dyspnea or other end points (e.g., fatigue, leg pain, angina, or certain ECG abnormalities) are reached (Blomqvist, 1971). The ECG is recorded and monitored by a physician both during exercise and for six minutes afterward with the subject in the sitting position. Heart rate is estimated from the ECG. Blood pressure is measured at two-minute intervals after exercise; since 1976, it has also been measured during each stage of exercise. All ECGs are evaluated by the Minnesota Code (Rose and Blackburn, 1968).

Stress thallium scans. Since November 1977, myocardial imaging during exercise has been employed in all subjects above the age of 40 years who consent to the test. The limitation to persons over 40 was imposed because of the exposure to radiation. The thallium scan is a relatively new non-invasive technique designed to assess abnormalities in myocardial perfusion at rest and during exercise. A small amount of ²⁰¹thallium is injected intravenously during the final minute of maximal treadmill exercise while simultaneous multi-lead ECGs are recorded. Myocardial scans in multiple projections are made with a gamma camera linked to a computer-based display and quantification system. Redistribution scans are made two hours after the conclusion of exercise. The records, after having been read subjectively by two independent observers, are maintained for later computer processing and objective quantification. The studies were initiated in collaboration with The Johns Hopkins University and are performed at the Johns Hopkins University Hospital (Lakatta, 1978).

2. Evaluation of the Effect of Age on Cardiovascular Function

In those individuals who show no evidence of cardiovascular disease, many aspects of cardiovascular function are evaluated at rest and during stress.

Systolic time intervals. A number of time intervals during the cardiac cycle have been related to myocardial performance (Weissler et al., 1969). Simultaneous recording of the ECG, phonocardiogram, and carotid pulse made it possible to determine the duration of electro-mechanical systole, left-ventricular ejection time, and the pre-ejection period. The protocol was discontinued in 1972 with the advent of echocardiography.

Echocardiography. The echocardiogram, devised to estimate cardiac performance, records the reflection of ultrasonic waves directed toward the heart from an external probe. The record obtained by this non-invasive technique permits measurements of left-ventricular wall thickness and cavity dimension, filling and ejecting rates, ejection fraction, and left-atrial and aortic-root diameters. One-dimensional echocardiograms are recorded with the subject either supine or rotated into the left lateral position. It is anticipated that echocardiographic testing will be repeated at seven-year intervals.

Since January 1978, echocardiograms with simultaneous recording from two probes, to permit greater accuracy in the calculation of volumes of the heart chambers and detection of regional dysfunction, have been recorded in randomly selected subjects. In addition to measurements at rest, observations are also made during

standardized semi-supine bicycle exercise. A semi-automated computer-assisted system quantifies structural and functional features of the left ventricle at rest and during exercise (Van Tosh et al., 1980). Measurements include myocardial and chamber areas and derived indices of left-ventricular mass and volume, mean and maximal velocity of fiber shortening, rates of diastolic lengthening, time intervals within the cardiac cycle, and regional indices of myocardial function, including left-ventricular thinning and thickening rates. This test is administered to all participants who volunteer and is supervised by the staff of the Division of Cardiology of the Department of Medicine, The Johns Hopkins University, in collaboration with the GRC Cardiovascular Section.

Multi-gated cardiac blood-pool scans (MUGA). Gated radionuclide angiography is a non-invasive method that has provided accurate and reliable estimates of left-ventricular ejection fractions both at rest and during exercise. A recent modification of the technique permits accurate measurements of left-ventricular volume throughout the cardiac cycle. It is thus an ideal technique for examination of left-ventricular function in normal human volunteers.

The protocol for the gated cardiac blood-pool scan is as follows: 2 cc of "cold pyrophosphate" are injected intravenously; after 20 minutes, 12 millicuries of technetium 99m per square meter of body-surface area are injected and supine resting gated cardiac blood-pool scan is performed. The subject then engages in graded upright bicycle exercise. Hematocrit is determined before and after maximal exercise; after exercise, a 10-cc blood sample is drawn and counted by the camera and a static marker image obtained to determine the distance between the center of the left ventricle and the chest wall. Computer analysis of the acquired images calculates left-ventricular ejection fraction, end-systolic, end-diastolic, and stroke volumes, and cardiac output for each stage of exercise. Regional wall motion is assessed visually and by computer methods (Rodeheffer et al., 1981). This test is performed at the Johns Hopkins Hospital in a collaborative study between the GRC and the Johns Hopkins Division of Cardiology.

Catecholamine secretion. At rest and during a graded treadmill exercise protocol in a subset of volunteers, oxygen consumption and plasma epinephrine, norepinephrine, and lactate were measured along with heart rate and blood pressure.

Heart-rate response to β -adrenergic stimulation. In a small subset of the population graded intravenous boluses of isoproterenol were injected in order to determine the effect of age on the heart-rate response to β -adrenergic stimulation. The end point of the protocol was an increment of 25 beats per minute over baseline.

Ventricular response to afterload stress. The left-ventricular response of young adult and old participants to hemodynamic stress was compared. Echocardiographic measurements of left-ventricular end-diastolic dimension, left-ventricular end-systolic dimension, and velocity of circumferential fiber shortening were made at rest and during 30-mm Hg increases in systolic blood pressure induced by handgrip exercise or phenylephrine infusion. In order to eliminate the influence of β -adrenergic drive, the measurements were repeated during β -adrenergic blockade with propranolol. The efficacy of the block was tested by demonstration that isoproterenol infusion did not result in an increase in heart rate (Yin et al., 1978).

Non-invasive His-bundle electrocardiography. Previous studies have shown an age-related increase in atrioventricular (AV) conduction time (PR interval) but have not localized its origin in relation to the His bundle. A microprocessor-assisted high-resolution ECG has been employed on selected subjects since January 1981. This

technique uses signal averaging and amplification of 512 cardiac cycles as well as filtration of random noise to record low-amplitude ECG signals from the body surface. From these recordings, a His-bundle potential can be identified in a substantial percentage of individuals. This allows an assessment of AV conduction both proximal to the His bundle (PH interval) and distal to it (HV interval) (Das et al., 1982).

Inotropic effect of digitalis. Because digitalis is commonly prescribed in the elderly and is attended by a high risk of toxicity, averaging 20% in most series (Beller et al., 1971), it is of vital concern whether its inotropic efficacy is decreased with advanced age in man. To answer this question, one-dimensional echocardiography and systolic time intervals have been used to measure the effect of an intravenous bolus of ouabain, a rapidly acting digitalis glycoside, on cardiac performance in healthy men. After baseline measurements are obtained, β -adrenergic blockade is induced by intravenous propranolol in order to eliminate variations in resting sympathetic tone that could obscure the effects of digitalis. Echograms and systolic time intervals are obtained periodically for 90 minutes after injection of ouabain.

Ambulatory ECG and blood-pressure monitoring. Since July 1978, an ambulatory 24-hour ECG has been recorded in normal BLSA participants during their routine activities at the GRC. The two-channel ECGs are analyzed by a semi-automated computer technique for heart rate, ectopic beats, and disturbances in conduction. Thus far 150 participants, all of whom were clinically healthy and had normal ECG responses to maximal treadmill exercise, have been studied by this technique.

Since January 1981, ambulatory ECGs and ambulatory blood pressure have been recorded automatically over a 24-hour period in normal subjects of all ages. Blood pressure is taken automatically every 7½ minutes and recorded on the same tape used to record the ambulatory ECG. This technique allows assessment of diurnal variability and absolute level of blood pressure as a function of age.

Peripheral blood flow. Venous-occlusion plethysmography was initiated in July 1980 to assess aging changes in maximum peripheral blood flow to the leg. By this technique the leg is placed in a water-filled box equipped to measure changes in limb volume. A blood-pressure cuff placed around the leg proximal to the plethysmograph is used to occlude arterial flow for periods of one to five minutes. The cuff pressure is then rapidly lowered to a level sufficient to occlude venous outflow but not arterial inflow. The consequent increase in leg volume, which is equivalent to arterial inflow, is then calculated from the rate of change in water pressure in the box with time. The procedure is initially performed at a water temperature of 27°C; it is repeated at 32°C to assess the increase in arterial flow generated by thermal stress. Such measurements allow assessment of maximal arterial flow and recovery rates from the induced limb ischemia. These values may then be correlated with known risk factors and predictors for the development of peripheral vascular disease.

CARBOHYDRATE METABOLISM: THE GLUCOSE-INSULIN HOMEOSTATIC SYSTEM

Tests to evaluate the glucose-insulin homeostatic system were given during the following periods:

Intravenous glucose tolerance test (IVGTT), January 1963-June 1977.

Intravenous insulin tolerance test (IVITT), June 1963-June 1964.

Cortisone glucose tolerance test (CGTT), January 1964-June 1977.

Oral glucose tolerance test (OGTT), 1.75 g/kg body weight, July 1964-June 1977; since June 1977 the glucose dose has been 40 g/m² surface area.

Intravenous tolbutamide response test (TRT), January 1965-June 1977.

One of the tests is usually performed on each visit. The four tests that were administered until June 1977 were generally given in a series that was repeated after all four had been administered. Since that date only the OGTT has been done. All are performed under basal conditions.

Activity prior to the test is limited, since subjects spend the preceding night at the Center as part of their 2½-day stay. Subjects remain in bed, in a reclining or a semi-reclining position, during the test. Smoking is not permitted before or during the test. Fasting and all subsequent venous-blood samples are obtained through an indwelling catheter.

Glucose was determined by a manual glucose oxidase method until September 1963; by the AutoAnalyzer automated ferricyanide reduction method (Technicon Instruments Corp., Tarrytown, New York) until June 1977; and thereafter by an automated glucose oxidase method (Beckman Instruments, Inc., Fullerton, California). Initially, from 1963 to 1966, whole-blood samples were deproteinized by the Somogyi technique (1945). Since 1966, plasma samples have been analyzed without deproteinization. Factors for conversion from the manual glucose oxidase to the automated ferricyanide method and from whole-blood to plasma samples were determined by simultaneous analyses of multiple specimens by the older and the newer methods. Since the AutoAnalyzer and Beckman methods gave nearly identical results, it was not necessary to apply a conversion factor.

The tests have been performed as follows:

Intravenous glucose tolerance test (IVGTT). A 20% solution of dextrose in water (0.375 g/kg body weight) was infused at a constant rate over a five-minute period. Blood samples were collected every ten minutes until 60 minutes; in the earliest studies, a final sample was collected at 80 minutes.

Intravenous insulin tolerance test (IVITT). A dose of 0.05 units of crystalline zinc insulin per kg body weight was injected nearly instantaneously. Venous-blood samples were collected from an indwelling catheter at ten-minute intervals for one hour.

Cortisone glucose tolerance test (CGTT). Cortisone acetate was given by mouth 8.5 and two hours before glucose ingestion, in two equal doses determined by body weight (< 124 lb = 37.5 mg; 124–159 lb = 50 mg; 160–194 lb = 62.5 mg; 195–230 lb = 75 mg; and > 230 lb = 87.5 mg). The glucose dose was 1.75 g/kg actual (as opposed to “desirable”) body weight, given as a 30% solution flavored with lemon juice, which was ingested in ten minutes or less. Blood samples were drawn every 20 minutes for two hours (Pozefsky et al., 1965).

Oral glucose tolerance test (OGTT). The technique was originally the same as that used for the CGTT, but the steroid administration was omitted. In July 1977 the glucose dose was changed to 40 g/m² surface area, in accordance with the recommendation of the Committee on Statistics of the American Diabetes Association (Klimt et al., 1969).

In 1979 the National Diabetes Data Group recommended that 75g of glucose be given to all subjects regardless of body size. This dose is equivalent to 40 g/m² surface area for an average-sized person. We have elected to continue use of the 40 g/m²

dosage, which the Data Group had rejected as impractical for the usual clinical test situations. Considering a dose adjustment for body size an advantageous detail, we decided not to introduce still another technical change into our study.

Intravenous tolbutamide response test (TRT). One gram of sodium tolbutamide in a 5% solution per 70 kg body weight was injected through an indwelling intravenous catheter in two minutes. Zero time was taken as the midpoint of the injection. Blood samples were obtained at 2, 6, 10, 15, 20, 30, 45, and 60 minutes.

Performance was judged primarily by the percentage of fall in glucose concentration at 20 and 30 minutes (Swerdloff et al., 1967).

Glucose-clamp test. A manual feedback technique, the glucose clamp, makes it possible to maintain blood glucose at any level chosen by the investigator (Andres et al., 1966; DeFronzo et al., 1979). Two basic types of study have been performed. In the "euglycemic clamp study," insulin is infused at a constant rate while the blood-glucose concentration is maintained at the subject's basal level. This is primarily a test of sensitivity of body tissues to insulin. In the "hyperglycemic clamp study," the plasma-glucose concentration is raised rapidly to a hyperglycemic plateau and is maintained at that level for two hours. The glucose plateaus studied have been 54, 98, 143, or 231 mg/dl above the basal level. This is a test of pancreatic beta-cell sensitivity to glucose and sensitivity of body tissues to insulin. These tests were given to subjects selected for special characteristics with respect to glucose tolerance and obesity. The schedule for retesting has not yet been determined.

METABOLISM OF DRUGS

1. Antipyrene Metabolism

Antipyrene, which is almost entirely metabolized by the liver, has been used as a marker drug for the hepatic microsomal enzyme system. A cross-sectional study of antipyrene metabolism was carried out between 1958 and 1967. Healthy subjects who were receiving no potentially interfering medication were included in the analysis of the interactive effects of age, smoking, consumption of caffeine, and alcohol intake. The subjects received an intravenous infusion of antipyrene (1 g/30 ml isotonic saline) in a 20-minute period. Plasma levels of antipyrene were measured at 0, 2, 4, 6, and 8 hours by the method of Brodie et al. (1949). The overall elimination rate constant (k_e) and the theoretical plasma concentration at zero time (C_0) for each subject were determined from regression analysis of the natural log of the plasma concentration with respect to time. Biologic half-life, apparent volume of distribution, and metabolic clearance rate were computed on the assumption of a single distribution volume and simple exponential decay by standard pharmacokinetic formulae (Vestal et al., 1975).

2. Ethanol Metabolism

In 1974-1975, a cross-sectional multidisciplinary study of ethanol metabolism and aging was undertaken (Vestal et al., 1977). Observations were made not only of ethanol kinetics, but also of plasma arginine vasopressin (AVP) response and reaction time and memory. The subjects, who had abstained from alcohol for three weeks, received a one-hour infusion of ethanol at a rate of 375 mg/m² surface area per minute

via an antecubital intravenous catheter. Blood samples for measurement of blood-ethanol concentrations were obtained at frequent intervals during the infusion and for four hours post infusion. Blood ethanol was assayed by a modification of the methods of Payne et al. (1967) and Roach and Creaven (1968). Ethanol kinetics were computed by the compartmental analysis of Berman and Weiss (1976). Body composition was calculated by the anthropometric method of Behnke (1961).

The effect of ethanol on plasma AVP levels (antidiuretic hormone) was assessed by radioimmunoassay (Robertson et al., 1973). Reaction-time and memory tests were carried out during and after the ethanol infusion by techniques similar to those described below under "Learning, Memory, and Decision Tasks."

HYPOTHALAMIC-PITUITARY FUNCTION

1. Reproductive Hormone System

This research, which was performed from 1977 to 1979, evaluated the hypothalamic-pituitary-testicular system, then correlated the endocrine studies with sexual history. Baseline plasma samples were obtained and a two-hour LHRH test was performed to measure pituitary gonadotropin reserve. This was followed by injection of human chorionic gonadotropin (hCG) to evaluate testis secretory reserve. A second injection was given the next morning. Blood samples were obtained after the injection of hCG and the following morning.

Plasma gonadotropins were assayed by double-antibody radioimmunoassay; plasma testosterone and dihydrotestosterone by radioimmunoassay; plasma estrone and estradiol by charcoal radioimmunoassay; and free testosterone by an ion-exchange technique (Harman and Danner, 1977; Harman and Tsitouras, 1980; Harman et al., 1980; S.M. Harman, 1981). Stored lyophilized plasma samples obtained at earlier ages were also assayed and their testosterone levels correlated with the sexual-behavior histories of the same subjects.

2. Hypothalamic-Posterior Pituitary Function

The effect of age on this endocrine system was determined cross-sectionally by assessment of the change in plasma-AVP levels in response to both a secretory stimulus (hypertonic saline) and an inhibitory stimulus (ethanol). The tests were performed from 1974 to 1976 in a small, carefully selected subset of the BLSA population.

Ethanol infusion test. In a subset of the subjects who were given ethanol intravenously, AVP responses were followed (see above, "Metabolism of Drugs," for a description of the ethanol-infusion protocol). Subjects abstained from all alcohol for at least 21 days before the study. AVP was measured by radioimmunoassay (Robertson et al., 1973). Samples of venous blood were obtained at short intervals during the ethanol infusion and for five hours thereafter (Vestal et al., 1977).

Hypertonic saline infusion test. Very careful screening of volunteers to exclude subjects with renal, hepatic, or cardiac disease was necessary. A two-hour infusion of 3% NaCl was given at a rate of 0.1 ml/kg body weight per minute after a 12-hour period of dehydration. Blood samples were collected every 20 minutes during the infusion (Helderman et al., 1978).

THE IMMUNE SYSTEM

Assessment of immune function is centered on the determination of serum antibody levels, serum immuno-protein levels, the function of peripheral blood lymphocytes *in vitro*, and the analysis of granulocytic cell function, also *in vitro* (tissue culture). The assays measure immune function in separate areas: the thymic-dependent area (cell-mediated immunity), the bone-marrow-dependent area (humoral immunity), and the area of nonspecific host resistance to infective organisms.

The several assays for cell-mediated immune function include proliferative response to a mitogenic agent, the ability to cooperate with antibody-forming cell-precursors to initiate an immune response, the enumeration of T lymphocytes and T-cell subset populations using morphologic criteria, and the measurement of lymphocytic ability to kill tumor cells.

The assays for the "B"-cell activity (humoral immunity), which include measurements of cellular activity, serum-protein concentrations, and morphologic identification, are based on the functional ability of some lymphoid cells to make antibodies. The assay for granulocytic cell activity measures metabolic activity during a period in which the cells are phagocytosing latex particles, as well as the ability of granulocytes to kill phagocytized bacteria.

The tests are carried out on leucocytes separated from fasting blood samples drawn from all subjects. The program, initiated in 1978, anticipates repetition of tests at six-year intervals (Adler et al., 1977).

CELL REPLICATION

Since previous studies had shown that the number of replications of human cells grown in culture is inversely related to the age of the donor (Martin et al., 1970; Schneider and Mitsui, 1976), a study of *in-vivo* human cellular aging in skin fibroblast cultures was introduced into the BLSA in 1974. Skin fibroblast cultures are established from a 2-mm punch biopsy obtained from the inner aspect of the left upper arm from male volunteers aged 17 to 96 years. In addition, biopsies are repeated at intervals of from three to five years after the initial sampling.

Cells are cultured under standardized conditions (Schneider and Mitsui, 1976). Initially, several observations were made on successive transfers of each culture: time of onset of senescent phase (failure of culture to reach confluency within one month of transfer), *in-vitro* life span of culture, cell-population replication rate, percentage of replicating cells in the culture, cell number at confluency, percentage of cells able to form large colonies, receptors for insulin and epidermal growth factor, prostacyclin synthesis, viral replication, and sister chromatid exchanges per cell. Subsequent observations have been more limited in their scope and include only culture life span, time of onset of cell senescence, and capacity for colony formation.

Cultures derived from each subject are frozen and stored at the GRC for direct comparison with other cultures taken from the same subject at a later visit. In addition, subcultures from 100 biopsies have been forwarded to the Aging Cell Repository, Institute for Medical Research, Camden, New Jersey, where they are now available for study by interested investigators.

SPECIAL SENSES

1. Eye Tonography

This test is administered to all subjects on each visit. A four-minute Schiötz test (Drews, 1967) is performed for each eye; intraocular pressure is increased by a 5.5-g pressure transducer placed on the cornea, which has been locally anesthetized by tropicamide, and the change in pressure is recorded over a four-minute period. Subjects with histories of corneal injuries are not tested.

2. Visual Screening Tests

Since 1964, the Titmus Optical Vision Tester has been used to evaluate visual function in all subjects (Titmus Optical Co., Petersburg, Virginia, 1959). The following observations are recorded: acuity (binocular, right eye and left eye, near and far); stereopsis (depth vision); color discrimination; vertical phoria; and lateral phoria, near and far. These tests were administered to each subject on alternate visits from 1964 to 1974; since 1974, they have been administered at each visit.

3. Fundus Photography

Since 1975, retinal photographs have been taken to identify vascular changes in the eye. Stereoscopic views of the macula and the optic-disc areas of the fundus of each eye are photographed in color with the Zeiss Fundus Camera. Since the procedure requires dilation of the pupils, subjects with histories of angle-closure glaucoma or Schiötz pressures (see above, "Eye Tonography") of 23 mm Hg or greater are not tested. This test is conducted at the GRC in collaboration with the staff of the Wilmer Eye Institute, The Johns Hopkins University.

4. Audiometry

Pure and pulsed-tone audiometric tests are performed with the Bekesy Audiometer, Model E800 (Bekesy, 1947; Corso, 1955; Hirsh, 1962). The test is administered with the subject seated in a soundproof cabinet. Tones are presented to each ear through air-conduction headphones. The subject's task is to depress a switch when he hears the tone and to release the switch when the tone disappears. The switch controls the motor-driven attenuator of the audiometer: When it is depressed, the signal intensity decreases; when it is released, signal intensity increases. A pen connected to the attenuator traces a continuous record of the subject's intensity adjustments on an audiogram form, producing a graphic representation of his threshold. Auditory thresholds are determined at pure tone frequencies between 150 and 8000 Hz.

Between 1965 and 1973 this test was administered to each subject on alternate visits; since 1974, it has been administered on every visit.

COGNITIVE PERFORMANCE

1. Intelligence Tests

The Army Alpha examination (forms A and B) is a combination of the five forms of Alpha used in the United States Army during World War I. The questions best for general use were selected by order-of-merit method. Items addressed specifically to male recruits were excluded and military terms modified (Bregman, 1925, 1947).

Form A of this paper-and-pencil examination, consisting of eight subtests, has been administered to all subjects since 1960. Form B is administered on visit 5 (six to eight years later), and Form A is repeated on visit 9; the two forms are given alternately on all subsequent visits. When the subject reaches age 70 and is tested every year, the form of the test given on the last previous visit is repeated. A speed score is obtained for each subtest by stopping the subject after the time specified, while a power score is obtained by permitting the subject to spend as much time as he likes in completing each test.

The Vocabulary Test (WAIS). This subtest from the Wechsler Adult Intelligence Scale (Wechsler, 1955) has been administered at six-year intervals since 1960. The task is to define 40 words.

Southern California Tests of Mental Ability. From 1959 to 1978 this battery of tests (Christensen et al., 1958; Christensen and Guilford, 1959) was administered twice, at a six-year interval. First-time testing was discontinued in 1972; by 1978, all participants who had taken the earlier test had completed it a second time, and the test was discontinued. The five timed tests (Associational Fluency, Expressional Fluency, Ideational Fluency, Word Fluency, and Consequences) were designed to measure several aspects of creative thinking. For example, in Expressional Fluency the task is to write meaningful four-word sentences in which the initial letter of each word has been specified; in Ideational Fluency, the subject lists items that meet such specific criteria as "fluids that burn."

2. Learning, Memory, and Decision Tasks

Verbal learning. Data collection began in 1960 for two studies of verbal learning. In serial learning, a list of familiar words is presented repeatedly in the same order. The task is to respond to each word with the next word in the list. In paired-associate learning, items that consist of a stimulus (two consonants) and a response component (a familiar adjective) are presented repeatedly in different orders. The task is to say the word that is paired with the two consonants. For both tasks, each subject was assigned to one of three pace conditions determined by the amount of time permitted for each response. Total errors and trials to criterion are the measures (Arenberg, 1967b; Arenberg and Robertson-Tchabo, 1977). These tasks are repeated at six-year intervals with different sets of words and consonants.

Benton Visual Retention Test. This non-verbal memory test (Benton, 1963) has been administered at six-year intervals since 1960. Form C is used for the first administration, Form E for the second, Form D for the third, and Form C again for the fourth. Each form is made up of ten designs with one or more figures; the task is to reproduce each design from memory after inspecting it for ten seconds. The primary measure is the number of errors in all ten reproductions (Arenberg, 1978).

Memory and decision tasks. In 1978 the following set of memory and decision tasks was introduced:

- Single-trial, immediate free recall (IMFR). Each of four lists consists of 12 familiar nouns. After the words are shown paced, the task is to report as many words as possible.
- Forward digit memory. The task is to recall, in order, lists of three to nine digits presented auditorily.
- Delayed memory. After each IMFR list and an interpolated task (forward

digit memory), one of two delayed memory procedures is administered. In delayed free recall, the task is to report as many of the words as possible from the previous IMFR list. In delayed recognition, the 12 words from the previous IMFR list and 12 distractor words are shown one at a time, and the task is to decide whether the word has already been presented.

- Dichotic listening. The task is to identify two digits presented simultaneously, one to each ear. Each set consists of 28 pairs.
- Decision tasks. These tasks require response to the visual presentation of designated digits under five different conditions. With the exception of the first task, the display is paced at a rate of one digit per second. The first task is to respond to the onset of a zero. The second is to respond to a specified digit. The third is to respond to any even or odd digit. The fourth is to respond to an even-odd or odd-even sequence of digits, and the fifth to respond to any two consecutive even or consecutive odd digits. Decision time and accuracy are the measures.

The same set of tasks, with different word lists, is to be repeated six years after the first administration.

3. Problem Solving

Logical problem solving. An experimental procedure was designed to measure effectiveness of reasoning. The apparatus consists of a display with six numbered and three lettered lights, each of which has an adjacent push-button, and a central light (G) that has no button. Each problem contains a set of logical relations indicated by arrows between lights. The ultimate task in each problem is to arrive at the outcome, G, via a sequence of inputs. The number of uninformative inputs is the primary measure. A set of logically identical problems is administered at least six years later. From 1962 to 1966, each problem was presented as a single task (Arenberg, 1974). From 1966 to 1974, for subjects who were administered these problems for the first time, each problem was presented in two parts to obtain independent measures of performance in analysis and synthesis.

Concept identification. The ability to identify concepts in the context of a problem-solving task is also evaluated. Each of 12 concept problems requires the identification of one or two "poisoned" foods. The subject selects "meals" consisting of four of the foods on a list, and the experimenter indicates whether that "meal" is fatal. The task is to identify the "poisoned" foods with as few "meals" as possible. The two primary performance measures are correctness of the identification and effectiveness in reaching a solution as indicated by the number of "meal" selections. Concept problem solving, initiated in 1967, is administered at six-year intervals.

PERSONALITY AND DEVELOPMENTAL CHARACTERISTICS

1. Personality

Guilford-Zimmerman Temperament Survey. A questionnaire consisting of 300 items provides an assessment of ten traits: General Activity, Restraint, Ascendance, Socia-

bility, Emotional Stability, Objectivity, Friendliness, Thoughtfulness, Personal Relations, and Masculinity (Guilford and Zimmerman, 1956; Guilford et al., 1976). Each subject is given the standardized instructions individually and completes the questionnaire during the remainder of his visit to the GRC. Until 1978, the test was administered every six years; since that time the interval has been 12 years.

Eysenck Personality Inventory. A standard measure of personality, yielding scores for Neuroticism and Extraversion, as well as a Lie scale. There are 57 items in a yes-no format.

NEO Inventory (Costa and McCrae, 1980c). A 145-item personality questionnaire that measures six traits in each of three broad domains of personality: Neuroticism, Extraversion, and Openness to Experience. Questions are answered on a five-point Likert scale: "strongly disagree," "disagree," "neutral," "agree," "strongly agree."

NEO Rating Inventory (McCrae, 1982b). Spouses of BLSA participants are asked to complete the NEO Rating Inventory. Measures of six traits in each of three broad domains of personality are obtained to provide an alternative to self-report measurement of personality (see above, "NEO Inventory").

Perceptual tests. In a one-to-two-hour session at the GRC, subjects are administered three psychological tests: the Thematic Apperception Test (TAT), in which subjects are shown a series of pictures and asked to tell a story about each; an Embedded Figures Test, in which subjects locate a hidden figure in a complex design; and the Holtzman Inkblot Test, in which subjects are presented with a series of inkblots and asked to tell what they call to mind. Ninety-six participants have taken these perceptual tests once since 1979.

Social desirability scale (Crowne and Marlowe, 1964). Thirty-three yes-no questions are asked to measure the subject's tendency to give socially acceptable answers. The scale has been used as a measure of the need for approval as well as of defensiveness.

Imaginal Processes Inventory—daydreaming. Aspects of daydreaming and related imaginal processes are determined for all participants by their responses to the 344-item Imaginal Processes Inventory developed by Singer and Antrobus (1963, 1972; Singer, 1975), as revised in 1970. The inventory is usually administered to single subjects or small groups. Each participant is given a brief definition of daydreaming and an explanation of the general purposes of the study. Completion of the inventory is self-paced and without supervision. Each item has five response options representing points on a continuum implying frequency or quality. A total of 28 scales are determined from responses to non-overlapping items. Each scale contains 12 items, except one that contains 20. The scales measure the content and structure of daydreaming by items drawn from intensive interviews; items are both specific (e.g., "I daydream about saving a drowning child") and general (e.g., "In my daydreams I feel guilty for having escaped punishment") (Giambra, 1977b, 1977-78). The scales have internal consistency and test-retest reliability (Giambra, 1974). The inventory was introduced into the BLSA in 1972-1973 and is repeated at six-year intervals.

2. Developmental Antecedents

Parent-child relations questionnaire (Roe and Siegelman, 1963). Subjects record their perceptions of relations with their parents when they were children. Fifty questions yield scores for casual-demanding, love-rejection, and attention dimensions. There are separate forms for son-mother, son-father, daughter-mother, and daughter-father.

Activities and attitudes questionnaire. The schedule and inventory entitled "Your

Activities and Attitudes" (Cavan et al., 1949) is given to each participant to be filled out without supervision during his first visit to the GRC and readministered at every fourth visit. The inventory is composed of three parts: background information, including general information about the participant and his earlier life; an activity inventory; and an attitude inventory.

The activity inventory provides eleven subscores in such areas as leisure-time and religious activities, intimate personal contacts, security, and health status. The attitude inventory deals with the personal aspects of adjustment. It contains eight groups of statements concerning health, friends, work, economic security, religion, and feelings of usefulness, happiness, and family.

STRESS AND COPING PROCESSES

1. Stress

Schedule of Life Events (SLE). This is a checklist, completed each two years, of recent potentially stressful events. Subjects rate their perceived stressfulness.

Daily events checklist. Subjects are asked to indicate which of a series of minor daily stresses and strains they have recently experienced, and to rate their pleasantness or unpleasantness.

Stress-and-coping interview. A 90-minute private interview is given by a psychologist or psychiatrist concerning history of stresses and coping efforts. The interview is videotaped, and standard ratings are made by the interviewer and another rater.

2. Coping and Defense Mechanisms

Coping self-interview. Participants are asked to nominate three events they have recently experienced: a challenge, a threat, and a loss. For each, they are asked if they used any of a set of 50 coping responses in dealing with the problem they have selected. In addition, they indicate whether the response helped to solve the problem or made them feel better.

Coping questionnaire. From among recent life events experienced by subjects, one target event is selected. Participants are asked to indicate which of 118 ways of coping they used in dealing with the event. Scores for 28 different coping mechanisms are derived.

Defense-mechanism inventory (Gleser and Ihlevich, 1969). A series of stories is presented, and subjects are asked to imagine how they would respond in thought, actions, feelings, and fantasy to the circumstances described. Five dimensions of defensive processes can be scored from the instrument.

3. Adaptational Outcomes

Well-being assessment sheet. This instrument, administered each two years, assesses psychological well-being, satisfaction with various areas of life, and overall evaluation of life.

Profile of mood states. Subjects indicate on this form the level of disturbance in seven moods: tension, anger, depression, fatigue, vigor, friendliness, and confusion. There is also a total mood score. Different forms allow for administration under "right now" or "in the past week" conditions.

MARITAL AND SEXUAL EXPERIENCE

Since 1967, interviews have been conducted with BLSA males concerning their current and past experience of marriage and sexual activity. Data are collected in a single two-hour session by a sociologist with extensive experience in such interviews (Martin, 1975). Over the years the refusal rate has varied between 2% and 3%. In all, 777 men have completed interviews.

Each interview follows a predetermined series of questions that have been memorized by the investigator; information is recorded in a code. Subjects are asked about the presence or absence of coitus, masturbation, nocturnal emission, and homosexual activity in their adult lives, the age of the subject at onset of each, and the frequency of their occurrence in relation to age and marital status. Since these behaviors account for nearly all male orgasmic experience (Kinsey et al., 1948), their combined frequencies constitute a measure of sexual functioning that is essentially unobtainable by other means. These frequencies, expressed in the interview as times per week, per month, or per year, are then converted into the number of sexual events falling into each five-year interval between age 20 and the time of report. Additional questions elicit information on sexual attitudes and reactions and characterize other aspects of the participants' marital, residential, religious, occupational, educational, military, and parental-home experience. None of the interviews has been repeated.