

Validation and Comparison of Eight Physical Activity Questionnaires

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Several questionnaires are available for assessing physical activity, but few of these instruments have been validated, particularly with respect to energy balance. Twenty-one healthy men 28–55 years old participating in a free-living, controlled feeding experiment completed eight widely used physical activity questionnaires. These were compared with measured caloric intake and resting energy expenditure during a period of stable body weight. Physical activity indices or daily energy expenditure estimates derived from the questionnaires generally increased with energy intake. The questionnaires were moderately well correlated with each other: interquestionnaire correlation coefficients ranged from 0.09 to 0.81 (median = 0.53). Correlations between the questionnaires and energy intake, which ranged from 0.13 for the Minnesota Leisure Time Activity instrument to 0.49 for the Harvard Alumni questionnaire, were higher than between the questionnaires and nonresting energy expenditure; that is, energy intake minus resting energy expenditure (correlation coefficient range 0.05–0.32). The Five-City Project questionnaire yielded an average estimate of total caloric expenditure that most closely approximated intake (96%). These data indicate that although estimates of individual energy expenditure or physical activity may be less than optimal, most of the questionnaires evaluated provide reasonable group means for these parameters. (Epidemiology 1990;1:65–71)

Keywords: caloric intake, energy expenditure, energy metabolism, exercise, questionnaires.

The importance of energy balance, and particularly its two major modifiable components, energy intake and physical activity, in the development of several chronic diseases has become increasingly evident in recent years. For example, obesity, which to a large extent results from energy intake in excess of energy requirements, is associated with increased morbidity and mortality from coronary heart disease, cancer, and diabetes mellitus (1–3). Cardiovascular disease, osteoporosis, and possibly breast and colon cancer are thought to have physical inactivity as one etiologic component (4–8). Given current trends in the prevalence of obesity and sedentary lifestyles in the US and elsewhere, these relationships will likely continue to gain both importance and attention in the future.

The primary components of total daily energy expenditure include resting (or basal) metabolism (approximately 50%–75%), physical activity (15%–40%), and the thermic effects of food ($\leq 10\%$) (9). Assessment of physical activity or energy expenditure is possible through a variety of methods developed for diverse purposes, ranging from movement monitors and activity questionnaires to the measured excretion of isotopically

doubly-labeled water (10–12). Few of these tools have been adequately validated, however, and even fewer have received any energy-based validation.

We had the opportunity to evaluate several physical activity questionnaires within the context of a free-living, human feeding study in which food intake was precisely measured and resting energy expenditure could be determined. The questionnaires were validated against both energy intake and the difference between intake and resting expenditure, and were compared with one another.

Methods

This investigation was conducted during February 1987 within an ongoing study of the metabolic effects of changes in dietary fat and fiber on bile acid metabolism, fecal mutagenic activity, and hormonal status in men. This was a 30-week crossover study conducted at the Beltsville Human Nutrition Research Center using 43 free-living, healthy male volunteers, ages 20–55 years. Most of the participants were employees of the US Department of Agriculture. Subjects received all their weekday meals in the cafeteria of the human study facility and were given prepackaged meals for weekends. Body weight measurements were taken daily throughout the experiment, and blood, urine, and fecal samples were obtained at baseline and throughout the study. Two diets, each consumed for 10 weeks, differed in two essential respects: the proportion of daily calories derived from dietary fat (20% in the low-fat diet and 40%

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TABLE 1. Comparison of Original Questionnaires Used in the Present Validation Study

Questionnaire (reference #)	Activity Type	Method of Administration	Time Frame	Measurement Scale	Population Tested
Harvard Alumni (13)	leisure	self-administered	7 days	kcal/wk	males 35-74, females 42-77
Pennsylvania Alumni*	occupational and leisure	self-administered	7 days (also usual and past year)	kcal/d	males and females
Five-City Project (14)	occupational and leisure	interview	7 days	kcal/d	males and females 20-74
Framingham (15)	occupational and leisure	interview	usual activity	index (kcal/d)	males 45-65, females 35-64
Health Insurance Plan (16)	occupational and leisure	self-administered	usual activity	index	males \geq 30
Baecke (17)	occupational and leisure	self-administered	usual activity	index	males and females 20-32
Lipid Research Clinics (18)	occupational and leisure (strenuous)	interview	usual activity	index	males and females 20-69
Minnesota Leisure (19)	leisure	interview	12 months	average or annual kcal/d	males \geq 25

* Dr R. S. Paffenbarger, Jr, personal communication.

in the high-fat diet), and the total daily dietary fiber content (40-50 g in the low-fat diet, and 15 g in the high-fat regimen). Total daily energy intake ranged from 2400 to 4000 kcal. Adjustment of this intake as necessary to maintain stable body weights was made in 400 kcal increments early in the parent study. Thus with few exceptions, the men remained at both one energy intake level and stable body weight throughout the study period. The present validation study took place during the latter half of the second experimental period (ie, approximately four months after the start of the overall study), and included subjects on both diets.

STUDY PROTOCOL

Twenty-one subjects, ages 28-55 years (median 36), volunteered to participate in the present investigation of physical activity questionnaires. Because only two subjects could have their resting energy expenditure measured on any given morning, the study spanned a four-week period. One day before the morning examination, which entailed resting energy expenditure and body composition measurements, the subject was given a packet of activity questionnaires (the order of the questionnaires was randomly varied among the subjects), along with verbal and written instructions for their completion that evening. During the morning of measurements, one of the investigators checked the questionnaires for errors and omissions, and these (if any) were clarified with the subject. While subjects were in the fasting state, resting energy expenditure and underwater

weighing measurements were obtained (described below).

QUESTIONNAIRES

Eight physical activity questionnaires that had been used in epidemiologic studies and could be readily self-administered, coded, and entered into a computer data base were, with one exception (see below), selected from the available literature. The original design and essential elements of most of these instruments have been previously reviewed (11) and are summarized in Table 1. Three of the eight questionnaires involved a seven-day recall: the Harvard Alumni (13), Five-City Project (14), and Pennsylvania Alumni (R. S. Paffenbarger, Jr, personal communication) surveys, the latter also including other fitness questions that were not specifically evaluated in the present study. Four instruments asked about "usual" activity: the Framingham (15), Health Insurance Plan of Greater New York (16), Baecke (17), and Lipid Research Clinics (18) questionnaires. To permit comparison with the one-week period during which data for energy intake, resting expenditure, and the other questionnaires were collected, the four were modified to refer to activity in the preceding week only (ie, "in the past week" rather than "usual activity"). Thus the seven days immediately preceding the day of resting expenditure and body composition measurements served as the reference activity period for all of the above seven questionnaires. One 12-month leisure time activity survey—the Minnesota Leisure Time Physical Activity Survey (19)—was used to give a

TABLE 2. Mean Physical and Other Characteristics of 21 Study Subjects According to Energy Intake

Factor	Energy Intake Level			All Subjects
	2400/2800 kcal/d	3200 kcal/d	3600/4000 kcal/d	3257 (89)* kcal/d
Subjects (no.)	5	9	7	21
Age (years)	34.6 (3.0)	41.4 (3.1)	34.8 (2.1)	37.8 (1.8)
Height (cm)	176.6 (2.3)	179.0 (2.9)	181.4 (2.7)	179.3 (1.6)
Weight (kg)	72.3 (3.4)	79.2 (4.6)	79.7 (4.8)	77.7 (2.6)
Lean body mass (kg)	58.8 (2.2)	64.0 (2.9)	65.4 (2.8)	63.2 (1.7)
Body fat (%)	17.6 (3.2)	18.2 (2.5)	17.2 (3.2)	17.7 (1.6)
Intake/weight (kcal/kg/d)	38.1 (2.5)	41.4 (2.2)	47.9 (3.8)	42.8 (1.8)
Resting energy expenditure (kcal/d)	1256 (49)	1385 (68)	1397 (64)	1358 (39)
Intake - resting energy expenditure (kcal/d)	1464 (77)	1815 (68)	2318 (76)	1899 (84)
(Intake - resting energy expenditure)/intake (%)	54 (2)	57 (2)	62 (1)	58 (1)

* Standard error of the mean in parentheses.

more comprehensive list of specific activities, despite the lack of comparability with the seven-day measurement period. The questionnaires were originally designed to be either self-administered (four) or used in interviews (four). In the present study, all questionnaires were tested in a standard, self-administered format to facilitate their possible use in epidemiologic studies.

Only three instruments permitted estimates of total daily energy expenditure (ie, from sleep and rest to vigorous activities) in kilocalories for the previous week (the Pennsylvania, Five-City, and Framingham surveys). One questionnaire yielded kilocalories per week (leisure only) (Harvard); one estimated annual leisure activity expenditure in kilocalories, which we converted to a daily basis (the Minnesota survey); and the others yielded only numerical indices of physical activity (Baecke, Health Insurance Plan, and Lipid Clinics questionnaires).

RESTING ENERGY EXPENDITURE

Resting energy expenditure, as determined by indirect calorimetry, was measured by means of a metabolic measurement cart (Sensormedics, Fullerton, CA) using an open-circuit canopy system. The subjects reported to the laboratory at 6 or 9 AM after a 12-hour overnight fast. Following a 30-minute period of bed rest, oxygen consumption and carbon dioxide production were measured

for one hour. Data from a stable 10- to 15-minute period were selected for each subject. Resting expenditure was calculated using the Weir equation (20).

BODY COMPOSITION

For the seven days preceding the metabolic measurements, weight was determined each morning on an electronic balance to the nearest 0.01 kg while the subject was wearing a preweighed lab coat and underwear, and nude weight was calculated. Height without shoes was measured to the nearest 0.1 cm with a stadiometer. Hydrostatic weighing was performed using the methods of Akers and Buskirk (21), with simultaneous measurement of underwater weight and residual lung volume (by nitrogen dilution). The Siri equation (22) was used to estimate lean body mass and percent body fat from body density.

DATA ANALYSIS

Simple means and standard errors of the means were computed, and Spearman rank correlation coefficients were generated using standard statistical software (23).

Results

Several characteristics of the study subjects according to level of energy intake appear in Table 2. (There was one subject at 2400 kcal, four at 2800, five at 3600, and two at 4000 kcal of daily intake.) Mean height, weight, lean

TABLE 3. Mean Daily Energy Expenditure or Index Value from Physical Activity Questionnaires by Level of Energy Intake for 21 Men

	Energy Intake Level			All Subjects
	2400/2800 kcal/d	3200 kcal/d	3600/4000 kcal/d	3257 (89)* kcal/d
Subjects (no.)	5	9	7	21
Questionnaire				
Harvard Alumni (kcal/wk)	1039 (368)	1936 (550)	2917 (1030)	2049 (431)
Pennsylvania Alumni (kcal/d) (kcal/kg/d)	3407 (138) 47.4 (2.0)	4387 (349) 55.2 (3.0)	4688 (197) 60.1 (4.1)	4254 (194) 55.0 (2.1)
Five-City Project (kcal/d) (kcal/kg/d)	2786 (248) 38.5 (2.6)	3234 (223) 40.9 (1.8)	3272 (238) 41.2 (2.1)	3140 (138) 40.5 (1.2)
Framingham (kcal/d) (kcal/kg/d)	2352 (168) 32.6 (2.0)	2780 (252) 35.0 (2.6)	3120 (196) 40.1 (3.8)	2791 (142) 36.1 (1.8)
Minnesota Leisure (kcal/d)	439 (116)	883 (406)	672 (201)	707 (186)
Baecke (index)	6.8 (0.7)	7.5 (0.7)	8.1 (0.5)	7.5 (0.4)
Health Insurance Plan (index)	14.6 (2.3)	18.3 (2.4)	17.0 (1.7)	17.0 (1.3)
Lipid Research Clinics (index)	0.60 (0.40)	0.67 (0.29)	1.29 (0.28)	0.86 (0.19)

* Standard error of the mean in parentheses.

body mass, energy intake per unit of body weight, resting energy expenditure, difference between intake and resting energy expenditure (ie, nonresting energy expenditure), and proportion of energy intake not accounted for by resting metabolism all generally increased with energy intake. In contrast, there was little relation between intake and age or percent body fat. The following parameters were highly correlated in this population: height and lean body mass ($r = 0.78$); body mass index (weight [kg]/height [m]²) and both weight ($r = 0.83$) and percent body fat ($r = 0.84$); and resting energy expenditure and height ($r = 0.65$), weight ($r = 0.73$), and lean body mass ($r = 0.78$). In addition, height was correlated with weight ($r = 0.52$).

Table 3 demonstrates that the mean daily energy expenditure and physical activity indices generally increased with energy intake. The three questionnaires that estimated daily kilocalorie expenditure based on one week of activity (the Pennsylvania, Five-City, and Framingham surveys) gave mean values between 31% below and 36% above the measured daily-intake categories. For the study group as a whole, the Five-City questionnaire resulted in estimates of energy expenditure closest to intake (mean percent of intake = 96). In contrast, the Framingham and Pennsylvania questionnaires

yielded estimates approximately 14% below and 31% above the average level of intake, respectively. Kilocalories per kilogram per day for these same questionnaires (ie, intensity of daily energy expenditure) also increased with intake. The Harvard, Lipid Clinics, and Baecke indices all generally increased with intake. Both the Minnesota and Health Insurance Plan instruments gave estimates of the average annual daily kilocalorie and activity index (respectively) that were highest for the middle intake group.

Correlations between the physical activity questionnaires and total energy intake (Table 4) ranged from 0.13 (Minnesota) to 0.49 (Harvard) and were, with one exception (Minnesota), higher than the correlations with nonresting energy (ie, intake minus resting expenditure). In general, the five index questionnaires demonstrated less of a difference between their correlation with intake and nonresting energy than did the three energy expenditure surveys. The Harvard, Pennsylvania, and Framingham instruments were the most highly correlated with intake. In contrast, interquestionnaire correlations were generally greater (range 0.09 to 0.81; median 0.53), and the three instruments providing estimates of kilocalories per kilogram per day were highly correlated with one another.

TABLE 4. Spearman Correlation Coefficients Between Physical Activity Index or Daily Energy Expenditure Intensity* from Questionnaires and Other Questionnaires and Energy Intake for 21 Men

Questionnaire	Intake	Intake - REE†	HA	PA	FCP	FRAM	MLTA	BKE	HIP	LRC
Harvard Alumni (HA)	0.49	0.32	1.00							
Pennsylvania Alumni (PA)	0.47	0.20	0.50	1.00						
Five-City Project (FCP)	0.35	0.10	0.09	0.54	1.00					
Framingham (FRAM)	0.43	0.24	0.36	0.72	0.45	1.00				
Minnesota Leisure (MLTA)	0.13	0.17	0.54	0.47	0.48	0.33	1.00			
Baecke (BKE)	0.38	0.21	0.56	0.59	0.16	0.57	0.36	1.00		
Health Insurance Plan (HIP)	0.19	0.05	0.53	0.77	0.40	0.75	0.52	0.78	1.00	
Lipid Research Clinics (LRC)	0.40	0.24	0.81	0.58	0.31	0.48	0.63	0.68	0.68	1.00

* Pennsylvania, Five-City, and Framingham as kcal/kg/d.
† REE, resting energy expenditure.

Discussion

The present investigation is unique in several respects. First, earlier studies assessed only one or two physical activity questionnaires (eg, Harvard and Baecke [24, 25]), while eight instruments were evaluated simultaneously in the present investigation, and most of these have not previously been compared with each other. Second, actual measured dietary intake provided a more valid standard on which to base assessment of individual energy consumption for comparison with activity questionnaires than other methods previously used, including dietary recall, history, or records. Third, measurement of resting energy expenditure allowed us to attempt correlations with energy expenditure above basal needs, which is what some of the questionnaires focus on, while body composition data facilitated detailed description of the study population. Our primary source of measurement error therefore rests with the physical activity questionnaires. Concurrence of the intake, resting energy, and physical activity assessments during one week eliminated the potential discordance owing to nonoverlapping measurement periods. Furthermore, although the present study involved only men (a similar investigation of women is currently being completed), a range of age, occupations (eg, laborers, graduate students, and office managers), and physical activity levels (eg, sedentary lifestyles to long-distance runners) was represented and provided a relatively heterogeneous study population.

Our investigation also had its limitations. Most importantly, our study size was restricted both by the relatively small number of participants in the parent study

and by the complexity of some of the study methods. A single week of activity and measurements may not have permitted the spectrum of activity afforded by longer periods (eg, one or two months). Although not directly applicable, data for the 12-month Minnesota questionnaire, which shows a range of 110–4100 kcal per day and a coefficient of variation of 120%, support this notion. Additionally, the use of energy increments of 400 kcal instead of more individually calculated energy requirements adds error (in theory) to our intake–expenditure comparisons, if one assumes that some reflexive compensation of either resting or physical activity energy expenditure did not occur. Stable body weights over the entire study period, and particularly late in the study when this investigation took place, argue against much error from this source. Self-administration of the activity questionnaires, even with prior detailed instruction and a brief interview, may have biased our findings in favor of less complex questionnaires originally designed for self-administration, and against those requiring an interviewer. There is evidence in the present data, however, that this bias was not important, since the questionnaires most strongly related to intake and nonresting energy in our study included both interview (eg, the Five-City survey) and self-administered (eg, the Pennsylvania questionnaire) formats. Finally, modification of the four “usual-activity” instruments to refer only to activity during the previous study week somewhat limits conclusions that can be drawn from the original questionnaires.

While most of the questionnaires included in the present study have been related to various health out-

comes (notably, cardiovascular diseases), very limited information exists concerning their validity (11). Estimates of activity from a few instruments have been associated with occupation, level of education, or body mass index (14, 17, 26), and these associations (eg, an inverse relation with education and body mass index) were thought to support the relation between physical activity and the questionnaire results. With respect to the two factors for which we have data, occupation and body mass index, we found similar associations; that is, higher physical activity estimates (total, job, and leisure activity) were observed for persons occupationally more active or of lower body mass index (data not shown). Four of the questionnaires—Framingham, Health Insurance Plan, Lipid Clinics, and Minnesota—have previously been related to some measure of cardiac fitness such as treadmill testing or heart rate (16, 18, 19, 26), while only three—Harvard, Baecke, and Health Insurance Plan (24, 25, 27)—have been compared with energy intake (ie, three-day recalls or seven-day records). Very low correlations with intake were reported for these latter comparisons: correlation coefficients of -0.03 to -0.31 for Baecke (24), -0.10 for Health Insurance Plan (25), and no correlation for Harvard (24, 27). In contrast, we observed stronger correlations with intake for these instruments ($r = 0.38, 0.19,$ and 0.49 , respectively). It is likely that the inclusion of only relatively young men, the concurrence of intake and activity measurements, and in particular the reduced measurement error for intake compared with reported dietary information used in prior studies contributed to the higher correlations we observed. It should also be remembered that our validation focused on energy expenditure and some of its components rather than on the self-reported frequency, intensity, and duration of physical activity; additional studies (eg, observational) are needed to validate the latter.

The questionnaires were originally developed for different populations and purposes and therefore are aimed at various aspects of physical activity. While most inquire about both work and leisure activity, two (Harvard, Minnesota) explore only the latter component. The Minnesota questionnaire was also the only one in our study that did not refer specifically to activity during the preceding week, and this difference likely contributed to its poor correspondence to intake or intake minus resting energy. These differences notwithstanding, however, we observed interquestionnaire correlation coefficients that were generally high, suggesting that the forms measure similar parameters. The three instruments that estimate total daily energy expenditure (Pennsylvania, Five-City, and Framingham) measure

not only time spent being physically active, but sleep, rest, and sedentary-activity time as well. Average activity or expenditure intensity values represent the sum of the product of time spent in various activity intensity categories (vigorous, sedentary, etc) and the "metabolic equivalent" coefficients recommended and commonly used for each of those categories. Multiplying these average energy expenditure intensities by body weight results in estimated daily expenditure, which should in theory approximate daily intake. [Previous studies have shown that measured caloric intake exceeds total energy expenditure by an average of 200–300 kcal per day (28).] This relation would explain why in the present investigation these questionnaires gave kilocalorie estimates that more closely approximated (in absolute terms) energy intake rather than intake minus resting energy. It was surprising, however, that most of the other questionnaires were also more highly correlated to intake than to intake minus resting energy. This finding indicates that they, too, are in some way reflecting components of energy expenditure aside from physical activity alone; for example, persons who are more active may have greater lean body mass and therefore higher resting and total energy expenditure.

Some of the present findings concerning energy expenditure estimated from the Pennsylvania, Five-City, and Framingham questionnaires reflect differences in the design of these three instruments. The "metabolic equivalent" coefficients used in these surveys range from 1.0 to 10.0 and were lowest for the Framingham instrument. This observation explains the lower overall intensity and kilocalorie expenditure estimates for the Framingham questionnaire. Also, the discrepant energy expenditure estimates for the Pennsylvania and Five-City questionnaires primarily reflect the greater number of hours claimed by the men for the vigorous and moderate activity categories in the Pennsylvania compared with the Five-City questionnaire. Questionnaire format, including the examples of activities provided as a guide on the forms and self-administration versus interview administration, may have contributed to the latter finding. Excess expenditure (ie, over and above intake) for the Pennsylvania instrument therefore resulted from the use of slightly higher coefficients as well as higher estimates of time in the moderate and vigorous activity categories. These findings highlight the importance of both the metabolic equivalent coefficients and the time assigned to each category, and demonstrate that some subjective interpretation of activity levels may have occurred.

We observed substantial variability of physical activity in this sample of men. The questionnaires evaluated

demonstrated only fair correspondence either to measured energy intake or intake minus resting energy expenditure, implying that they would not provide accurate estimates of individual energy expenditure or physical activity. In contrast, group means show a much better relation for most of the questionnaires evaluated, a finding that indicates that they should be adequate for deriving a ranking of individuals by physical activity, such as is commonly used in epidemiologic studies.

Acknowledgments

The authors would like to thank Drs Rachel Ballard-Barbash, Carl Caspersen, and Thomas Glynn for their helpful comments, and William C. Campbell and Janice Collins for technical assistance.

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Cell cycle dependent changes of chromosomes in mouse fibroblasts

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Received August 7, 1978

Accepted January 2, 1979

Chromosome structure — arrangement of centromeres — cell cycle

Mouse fibroblast interphase nuclei stained with quinacrine dihydrochloride show distinctive differences in their fluorescent characteristics analogous to those which we have already observed in human and Syrian hamster cells. These patterns reflect the position of any given nucleus within the cell cycle. The brightly fluorescent chromocenters in the mouse nuclei were found to be in absolute agreement with those stained by the C-banding technique, indicating that they represent centromeric heterochromatin. Furthermore, their number and size per nucleus were shown to vary in relation to the progress of the cell cycle.

Introduction

Chromosomes become individually visible only during the short mitotic period. However, several observations suggest a continuous chromosome cycle throughout interphase [3, 4, 13, 14, 15]. This paper reports further cytological evidence for changes in the conformational state and the arrangement of chromosomes in murine fibroblasts which span the entire interphase.

Material and methods

Laboratory mouse embryos of the Swiss strain were used to establish primary fibroblast monolayer cultures by routine procedures. Only cells from female embryos, checked by karyogram analysis, were included in this study. They were cultured direct on microscopic slides in Petri dishes during the second and third passage.

Fixation and quinacrine dihydrochloride (QDH) staining procedures were carried out according to Moser et al. [9]. By replacing the aqueous buffer by a xylene-diluted neutral mounting medium (G. T. Gurr), it is possible to enhance the fluorescent intensity of chromatin in QDH stained nuclei, which accounts for part of the observations which will be described.

For establishing the cell cycle stage of specific nuclei several fibroblast cultures were exposed to 3H-thymidine (1 μ Ci/ml) for different periods of time (1/2h, 2h, 3h, 6h, 7h, 10h and 13h) prior to fixation. Nuclei with specific fluorescent patterns were localized by use of the England-Finder (Graticules Ltd., London). These were then photo-

graphed and relocated for the identification of the labelling pattern by applying the standard autoradiographic technique using Kodak AR-10 stripping film.

For the characterization of the brightly fluorescent heterochromatic particles, several samples were treated with the C-banding technique utilizing a saturated barium hydroxide solution [2, 11].

The QDH stained preparations were photographed with a Zeiss photomicroscope adapted to incident UV fluorescence (HBO 200 watt mercury vapor lamp, exciter filter BH 12, barrier filter 53/44). Because UV-illumination of the QDH stained nuclei may impair subsequent C-staining, the photographic exposure was kept at a minimum (10 sec). Kodak Tri-X film was rated at 1000 ASA and processed in Acufine (Acufine Inc., Chicago, Ill.) developer.

Results

Changes of the general nuclear fluorescence during the cell cycle

Unsynchronized embryonic mouse fibroblast nuclei stained with QDH show the same diversity of fluorescent patterns as we found in human cells [9]. However, in the mouse, these nuclei differ in that they possess a high number of particles of very brightly fluorescent chromatin which are presumed to represent constitutive heterochromatin [11]. We refer to them as chromocenters.

Figure 1 shows a montage of such QDH stained nuclei in relation to their position in the cell cycle. This scheme has been constructed on the basis of analogy to the human situation [9] and its accuracy was independently controlled by successive autoradiography.

The small, usually paired, early G1 nuclei show a very bright overall fluorescence of the chromosome threads. As the cell progresses into G1, the general fluorescence becomes faint and the granulation of the chromatin more dispersed while only the fluorescent intensity of the chromocenters seems to be unchanged. Late in G1, the nuclei reach a state where most of the chromatin hardly fluoresces while the nucleolar fluorescence appears somewhat more pronounced.

In advanced S phase, the nuclei begin to show a coarsely granular fluorescent pattern with increasing filamentous connections of the appearing chromatin masses. The contraction of the fluorescent chromatin threads continues into G2 phase to ultimate chromosome condensation and mitosis. No

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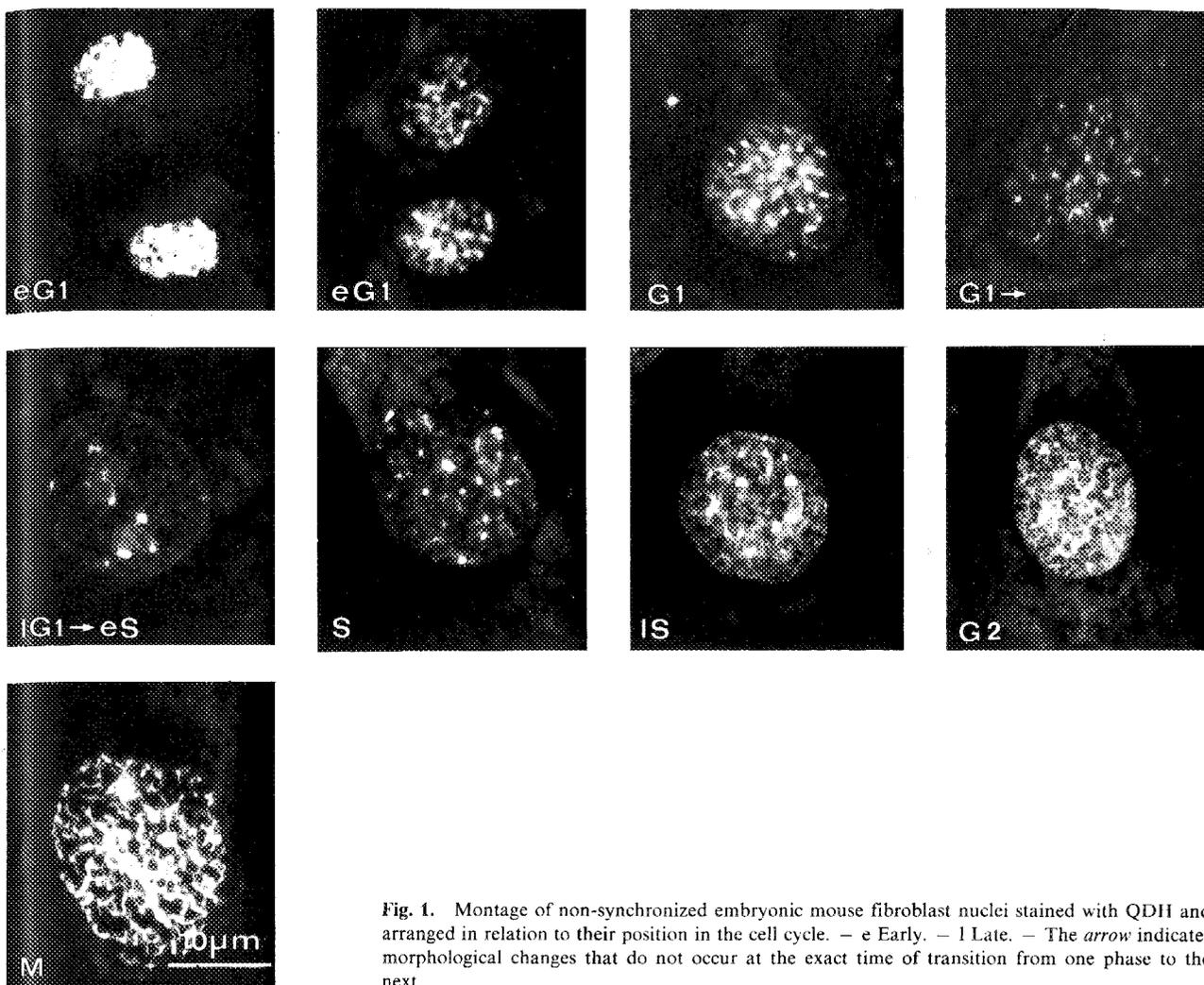


Fig. 1. Montage of non-synchronized embryonic mouse fibroblast nuclei stained with QDH and arranged in relation to their position in the cell cycle. — e Early. — l Late. — The arrow indicates morphological changes that do not occur at the exact time of transition from one phase to the next.

exceptions of this staining behavior could be found during the analysis of several hundred interphase nuclei of healthy mouse fibroblasts.

Characterization of the chromocenters

To resolve the question of whether all the brightly fluorescent chromocenters stain with the C-banding technique [2] which defines constitutive heterochromatin in metaphase chromosomes two cell preparations were consecutively stained with the QDH- and the C-banding method. 100 nuclei in all stages of the cell cycle were evaluated and absolute agreement was found in the appearance of the chromocenters: all particles stainable with the C-banding technique fluoresce brightly during the entire cell cycle (see Fig. 2). However, it is difficult to identify the chromocenters correctly before, during and immediately after mitosis because all the chromatin is brightly fluorescent at that time. No attempt was made to distinguish the X-chromatin from the constitutive heterochromatin, which is merged with the large number of other chromocenters and can only be recognized with certainty in a very small number of cells.

Frequency of chromocenters in relation to the cell cycle

All 40 mouse chromosomes are telocentric and, except for the Y chromosome, possess a recognizable segment of centromeric heterochromatin. Because such constitutive heterochromatin does not occur in other parts of the murine chromosomes, it serves as a useful marker to trace the location of the centromeres in interphase. As it has been previously shown [1, 7, 11], the number of chromocenters in interphase nuclei varies and rarely reaches the maximum of 40.

Table I represents data concerning the frequency of chromocenters in relation to the cell cycle stage. Nuclei in middle G1 and middle S have a similar fluorescent pattern and therefore were excluded from our evaluation. As seen in Table I a decrease in the absolute number of single chromocenters occurs during the course of G1. The lowest number is reached in late G1/early S when the rest of the chromatin appears dark. During late G1 and G2 the number increases to the expected maximum of 40 chromocenters as seen at the beginning of mitosis (Fig. 2).

There is not only a variation in the number of chromocenters but also in their size. Large chromocenters reaching a

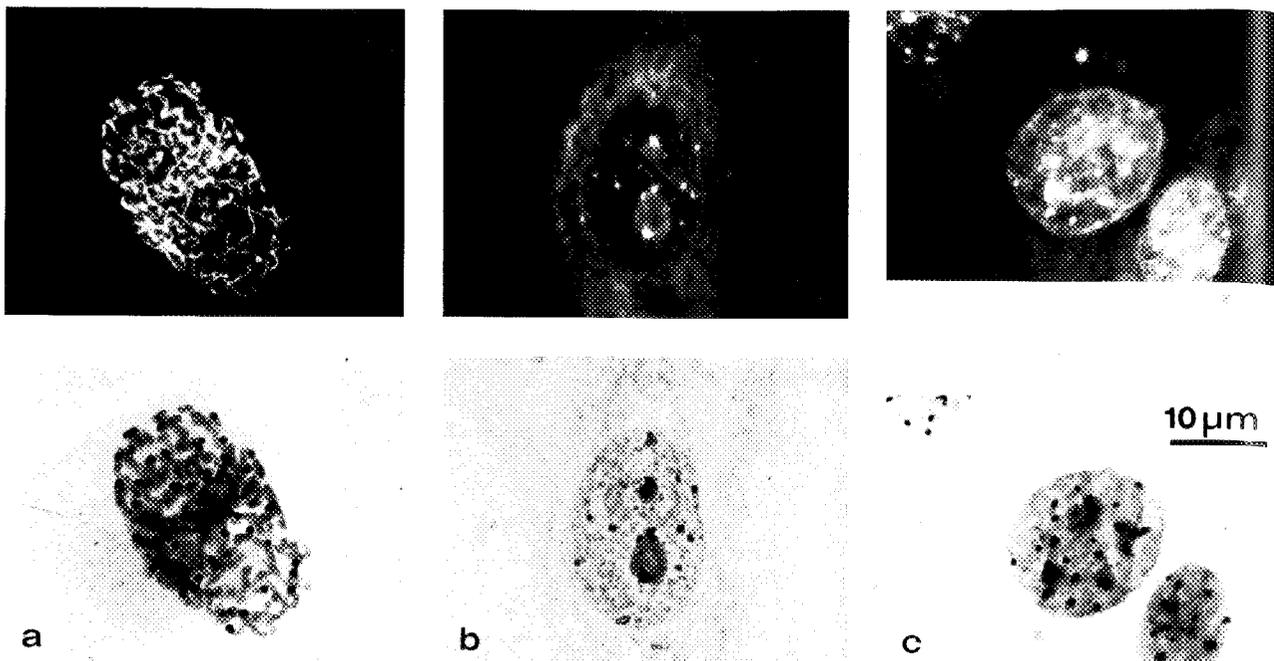


Fig. 2. Micrographs of nuclei. — *Top* Stained with QDH. — *Bottom* Stained with the C-staining method. — **a.** Tetraploid prophase cell with over 70 visible chromocenters. — **b.** Late G1 → early S nucleus; about 13 chromocenters visible. — **c.** Late S phase nucleus; about 20 visible chromocenters. — Note the precise agreement of the brightly fluorescent chromocenters on the top with the C-stained chromatin at the bottom.

diameter of 2 µm occur in late G1/S but are rarely noticed in G2. They are preferentially located at the nucleolus (see Figs. 1, 2).

The average number of nucleoli was found to be 2.3 per cell on the basis of 100 nuclei in the late G1 stage. The number of chromocenters attached to the nucleolus ranged from 1 to 5 with an average number of 3.

Tab. I. Frequency of chromocenters per nucleus in relation to the cell cycle stage. Results from 256 nuclei.

Cell cycle stage	Number of nuclei	Number of chromocenter average range	
Very early G1	12	> 25 ^{*)}	
Late G1/early S	186	13.8	3-20
Late S/G2 nuclei	43	18.6	11-27
Late G2/prophase	15	35.7	26-40

^{*)} Because of the high degree of condensation the exact number of chromocenters could not be determined.

Discussion

The reported observations provide further conclusive evidence for the existence of a continuous chromosome cycle during interphase [8]. With a modified QDH staining procedure, changes of chromosome architecture and arrangement could be visualized in mouse fibroblast nuclei that span the entire intermitotic period.

In general, the chromatin fluoresces most brightly during mitosis. A decrease of the overall fluorescent intensity begins in the early G1 phase. During late G1 and early S phase, the cells reach a stage where the chromatin shows practically no fluorescence. At that time, the DNA is particularly accessible to polymerase. The increase of fluorescence begins during the S phase and continues progressively throughout the G2 phase towards mitosis. Since the mouse chromatin shows analogous changes of fluorescent properties as already observed in human [9] and Syrian hamster cells [10] it can be assumed that they reflect the general behavior of mammalian chromatin. However, mouse nuclei differ in that they contain numerous brightly fluorescent particles in all cell cycle stages [11]. In our study we followed the arrangement of these chromocenters throughout interphase. This was possible, because the main fluorescent characteristics of the chromatin in a particular nucleus permits an identification of its position in the cell cycle. If additional criteria of pairing, shape and size of the nuclei are also considered than the accuracy of this procedure can be controlled.

There is already sufficient evidence to conclude that the constitutive heterochromatin is represented during interphase by the so-called chromocenters. Yaminesh and Yunis [15] separated the chromatin of mouse cells into condensed and dispersed chromatin fractions and found that the former contains most of the mouse satellite DNA. By hybridizing radioactive nucleic acid with the DNA of cytological preparations, Pardue and Gall [12] confirmed that the sequences of

the satellite DNA are located not only in the centromeric regions of metaphase chromosomes but also in the chromocenters of murine interphase nuclei. T. C. Hsu [6] and others demonstrated that the chromocenters have little or no RNA-synthetic activity. We applied the C-banding technique [2] to interphase nuclei in different cell cycle stages. The same nuclei had been previously investigated and photographed after QDH-staining. By comparing the chromocenters stained with both techniques we found that all the particles stainable with the C-banding technique also fluoresce brightly during the entire cell cycle. The fact that the centromeric heterochromatin fluoresces only weakly in hypotonically treated mouse metaphase chromosomes, but fluoresces brightly in interphase and mitosis if fixed and stained by our method indicates that the fluorescent intensity of specific chromosome segments can be modified by preparation procedures.

It has been reported that the number of chromocenters varies in interphase nuclei of the mouse [1, 7, 11] and that this number is different from tissue to tissue, but relatively constant in the cells of a common origin. By counting the number of the chromocenters in the nuclei of specific cell cycle stages (see Tab. I) we found strong evidence to conclude that the murine fibroblast nuclei go through an entire gamut of organizational differences of chromosomes. As the cell progresses in the cell cycle it reaches a stage late in G1 where the absolute number of chromocenters is lowest (average number: 13.8) in comparison with the expected 40 individual granules representing the heterochromatic regions of each chromosome (see Tab. I). At that stage, some of the chromocenters seem to be increased in size. Therefore, it can be concluded that the reduction of the number of chromocenters is caused by aggregation of some of them during interphase. However, it may be possible, that some mouse chromocenters undergo decondensation and can no longer form stainable heterochromatic granules. Unfortunately, the mouse heterochromatin is very uniform and it is not possible to follow specific heterochromatic segments in interphase as it is in human cells. During the late G1 phase some of the larger chromocenters are attached to the nucleoli which themselves vary in number from 1 to 5 per nucleus. These chromocenters most likely represent the heterochromatic region of the rDNA carrying chromosomes [5].

Acknowledgements. The authors are grateful to K. Moser and to W. Lüthy for their help with the photography.

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