Decisive Factors in Designing the Sudbury Study of Chronic Disease

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ASSESSING the prevalence of chronic disease by means of epidemiologic techniques can appear deceptively simple. The obvious difficulties of such studies are in the selection of the study population and its careful enumeration, the adequacy and validity of sampling, and the accurate assessment of nonresponse. Many of these factors have been discussed in relation to the experience gained by the Oxford diabetes study (*1*). Initiated in 1946, the Oxford study (*2*) was an attempt to use an entire community to gain information about a chronic disease. In this paper, however, we shall focus on the added problems encountered in studying chronic disease with measurements presumed to be objective. The design of the Sudbury, Mass., study (*3*) of diabetes mellitus, gout, and rheumatoid arthritis will be used to demonstrate possible ways of handling such problems.

Selected Sources of Variation

The variables selected for discussion are grouped in two large categories. The measurement fluctuations that occur within and between persons are considered under biological variation. The problems associated with the measurements chosen for study are considered under technical variation. Finally, the Sudbury study and the attempts to overcome some of these sources of variation are described. This description includes an outline of the design, considerations anticipated in handling the resulting data now being analyzed, and a description of the quality control procedures adopted.

Effect of Biological Variation

While the use of simple, valid measurements to diagnose a chronic disease will always be desirable, it would be unusual for any single biochemical measurement to be an absolute arbiter of health. For example, a sufficiently high blood sugar level will almost always indicate diabetes mellitus, yet no single blood sugar determination will segregate the diabetic population (*4*). For this reason, confirmatory retesting, in the form of repeat blood sugar measurements or glucose tolerance tests, is common for patients who screen positive, that is, those who have an initial blood sugar above a predetermined "high yield" or suspect level.

It is seldom recognized that such retesting must contend with the considerable fluctuation of biological values that occurs within the person, even under standardized conditions. This fluctuation will influence the final results, since the first test will usually find persons at various levels above or below their characteristic norm. The application of the second test consequently will be biased since persons who are above their

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usual norm will be tested while others who are below their norm will remain untested. For example, preliminary data from the Sudbury study indicate that 0.8 percent of persons have elevated postprandial blood sugars and an abnormal glucose tolerance test, while 1.2 percent are found to be abnormal when a random sample of the whole population is tested. Such losses from "screening" are reluctantly acceptable in detection drives as long as there is a satisfactory yield (*5*), although they are unacceptable to the descriptive epidemiologist who requires precision for accurate prevalence figures.

From the same example it can be seen that the bias resulting from selective retesting can be compensated for by establishing a corrective factor. When the diagnostic criterion, in this instance the glucose tolerance test, is performed on a random subsample of the population as well as on those who screen positive, the glucose tolerance values in the whole population can be estimated regardless of the result of the initial screening for blood sugar. This corrected prevalence rate should then be reproducible when retesting, despite individual fluctuations. Conclusive evidence of such rate stability is not available but is a logical extension of data derived from repetitive glucose tolerance testing at short intervals in a large group of prisoners. The findings indicated stability of both the mean blood sugar and the variation about that mean, despite remarkable intra-individual variations (*6*).

The possibility of obtaining reproducible population data does little to remedy the problems of the individual. While the same number of persons will rate abnormal when the whole study population is retested after a short interval, there is disagreement as to the particular persons identified. It is therefore of considerable importance to determine if persons with a normal test following a diagnostic test are normal persons manifesting a normal variation or whether they have the disease in question but it is now in remission. This distinction is necessary not only because remissions occur more frequently than is generally recognized but because they are often ascribed to treatment without adequate justification (*7*). More basically, the distinction is necessary for a correct interpretation of the diagnostic test for clinical application. For the epidemiologist, then, the critical impact of variability is in the development of incidence data since disease incidence requires the enumeration of persons in the population who develop the disease within a specified time interval.

Obviously, the frequency and duration of fluctuations from normal to abnormal can greatly affect incidence figures, particularly if the numbers of persons reaching defined abnormal levels accumulate. One study of rheumatoid arthritis consequently indicated that incidence depended on the frequency of testing (*8*). Table 1, abstracted from these data, indicates a variation from 4.2 to 10.5 percent, depending on whether one or six examinations were used to derive the results. From this study, Beall and Cobb (*8*) proposed the "proportion of time in episode" as a desirable measure of the frequency of a remittent disease. Nevertheless, the validity of this measure is still dependent on arbitrarily selected diagnostic criteria, and its value is consequently limited by the confidence in these criteria. Continued progress in understanding the natural history of chronic disease, for this reason, has not brought us much closer to a universally acceptable means of accurately determining simple prevalence and incidence rates.

Study and enumeration of persons with chronic disease are plagued by the very gaps in knowledge that are being investigated. One of the unparalleled values of an epidemiologic study is the establishment of a valid baseline that allows a value judgment of the initial diagnostic levels from the experience gained by continued observation (*4*). In this way, for example, the "defined" diabetic or rheumatoid person can be removed from an area of diagnostic doubt by the later development of an undisputed level of decompensation or deformity.

Effect of Technical Variation

Observer and laboratory variations further complicate the evaluation of epidemiologic studies. It is important both to measure and to minimize such technical problems by adequate controls. Otherwise, the evaluation of true biological variation will be impossible and interstudy comparisons greatly hampered.

The effect of using different methodologies is

Table 1. Probable rheumatoid arthritis (ARA classification) related to frequency of examinations [1]

Number of examinations	Examination frequency, months	Cumulative prevalence, percent
1	12	4.2
3	4	8.2
4	3	8.9
6	2	10.5

[1] Reference 8.

Table 2. Comparison of two blood sugar methods

Blood sugar method (venous whole blood)	Number of samples	Mean ± SD (mg. per 100 ml.)	Mean of differences ± SD (mg. per 100 ml.)
Somogyi-Nelson	287	89.9 ± 24.5	0.2 ± 6.7
AutoAnalyzer	287	89.8 ± 23.2	

often quite obvious. For example, Folin-Wu blood sugars will average 20 mg. per 100 ml. higher than those obtained by the Somogyi-Nelson method (*9*). On the other hand, two blood sugar methods giving remarkably close average results may mask classification differences that can be ascribed directly to the variability of the tests. Comparisons of a random set of the Sudbury blood samples illustrate this point (table 2). A mean difference of 0.2 mg. per 100 ml. confirmed the proximity of AutoAnalyzer (*A*) and Somogyi-Nelson results. The large standard deviation of the differences between methods, however, reflects the degree to which variations in the classification of persons depend on the method used.

Furthermore, the use of automated procedures for testing whole blood samples can produce differing results that remain undetected by standard quality control procedures. A haphazard sample of 48 blood samples from Sudbury were stored at −20° C. following glucose determinations with the AutoAnalyzer, which showed them to have a mean value of 109 mg. per 100 ml. Later repeat assays averaged 118 mg. per 100 ml., while accompanying aliquots from a serum pool of known glucose content failed to show a similar change in glucose recovery. This difference of 9 mg. per 100 ml. can be ascribed to hemolysis of the specimen caused by the freezing and thawing. The important lesson in this example is, first, that such differences cannot be identified by the usual quality control procedures and, second, that various degrees of hemolysis can go unnoticed in fresh unstored blood, giving rise to an unrecognized variation in the results.

Another study showed an average difference of 7.8 mg. per 100 ml. in results from two laboratories that were sent aliquots of the same sample, even though the identical method was used for both tests (*10*). The magnitude of the effects of such technical variation can be judged by the results of screening the 1960–62 population (*10*), using a critical blood sugar level of 150 mg. per 100 ml. A second laboratory could have screened 6 million more people as positive, using the identical laboratory procedures on the same samples, if it had a constant difference of 10 mg. per 100 ml. in results. Consequently, a difference of 10 mg. per 100 ml. which might be meaningless in many clinical situations, becomes critical for the epidemiologist.

It is all-important, therefore, to measure both the technical accuracy and the variability by a quality control system and, where possible, to tailor the control system to the character of the sample. Since chemical determinations are subject to a greater variation in results over a period of time, it is of great importance to measure the variability in a way that will reflect its extent throughout the study. To standardize the procedure, such a system should include operating guidelines for the technician. Further, an objective or "blind" measure of the accuracy and reproducibility of the method is obviously needed to avoid translating purely technical effects into inaccurate prevalence or incidence rates.

The Sudbury Study

The epidemiologic study in Sudbury, Mass., which involved aspects of diabetes mellitus, rheumatoid arthritis, and gout, was designed with the aforementioned problems in mind. These problems, far from being academic, have a direct bearing on the lack of agreement in the current literature. For example, figures for diabetes mellitus suggest a prevalence (*2*, *10*, *11*) varying incredibly from 2 to 16 percent, even when the differences in methodology were equated and testing included samples from the same general areas (*10*). Similarly, disagreement in studies of rheumatoid arthritis is implied in the work of Beall and Cobb (*8*), where the prevalence of probable rheumatoid arthritis (American Rheumatism Association or ARA classification) varied from 4.2 to 10.5 percent, depending on the number of examinations performed.

Current efforts at standardization have failed to eradicate many sources of variation among studies. Work in our laboratory, for instance, indicates that differing results can be obtained with tests for the rheumatoid factor although the criteria used are those recommended for epidemiologic studies (*12*). Studies in other laboratories also have implied that a serious potential exists for significant technical variation in determinations of uric acid (*13*).

A further problem is created by the customary method of separating persons with previously known or stated disease from those with disease discovered during the study. The acceptance of stated disease has now been definitely implicated as a source of error. Even in carefully conducted studies, later observations may fail to confirm the presence of disease in some of these persons (*10*, *14*). Consequently, particular attention must be paid to documenting the presence of disease in those persons with stated diabetes, rheumatoid arthritis, or gout.

Outline of study design. The essential features of the Sudbury health study design were as follows:

1. Testing within a 3-month period all persons aged 15 or older, or approximately 6,000 people. This test consisted of a medical history, a physical examination, a venous blood sample, and a urine specimen. The physical examination included an examination of joints and blood pressure, cardiographic, and anthropomorphic measurements. Whole blood in ethylenediamine tetracetic acid fluoride tubes was used for blood sugar tests; and serum without additive was used for uric acid, cholesterol, sheep cell agglutination, and bentonite flocculation tests. The urinary glucose, protein and ketones were obtained by semiquantitative methods, and the specimen was stored at $-20°$ C. for later quantitative testing.

Persons above predefined levels of positivity on initial testing were retested by postprandial blood sugars and glucose tolerance tests or by X-rays of the hand as appropriate. All persons with stated disease were required to have documented confirmation.

2. Glucose tolerance tests and hand X-rays of a random sample of the population, regardless of the inital study test results.

3. Assessment of nonresponse by questionnaire, telephone, and canvass of local physicians and hospitals.

4. Repeating, after 1 year, all the procedures in No. 1 on an age-stratified sample of the population.

5. Continued periodic examination of (*a*) persons in the age-stratified sample and (*b*) all additional persons screening suspect or abnormal on the initial tests.

Considerations in data handling. It was recognized at the beginning of the Sudbury study that certain limitations would bear on the data, particularly when more than one disease was being studied in a community setting. The time limitations set for the study and the multiple diseases that were included prevented all the initial samples from being obtained under rigidly standardized conditions. Preliminary testing of methods before the study was started, however, confirmed that the blood sugar determination would be the only one significantly affected by the previous meal.

Hyperglycemia and diabetes. To compensate for the varying postprandial intervals and to establish screening levels, recourse was made to the Oxford study data (*1*, *2*). The 90th percentile values were obtained for several post cibum intervals, and adjustments made for technical differences. Sudbury participants with blood sugars exceeding the resulting appropriate 90th percentile values were recalled for repeat measurements of blood sugar. Patients with repeat values still above these levels were challenged with a 100-gm. oral glucose tolerance test. It was recognized that the percentile values found for the Sudbury data might be different from the original Oxford values. Since this proved to be true, the final analysis will require appropriate modification if the Sudbury and Oxford data are compared.

A 5 percent random subsample of the population 15 years of age and older were invited to have a full glucose tolerance test. In this way it was possible to have a corrective estimate of the number of persons with diagnostic glucose tolerance tests that would compensate for the bias introduced by postprandial screening. This in turn will provide valid prevalence figures for newly discovered diabetes and allow evaluation of the disparity reported to exist between postprandial and postglucose blood sugar levels. Further, it will provide information on the distribution of blood glucose values for a population in terms of both postprandial blood sugars and standard glucose tolerance tests. Since much current disagreement exists on the norms for the glucose tolerance test, these distributions and their relation to future diabetes as determined by the progression of the study will prove invaluable.

Rheumatoid arthritis. For rheumatoid arthritis, the establishment of baseline data followed strict ARA criteria (*12*). The accomplishment of examining 77 percent of the population within a 3-month interval made it possible to obtain a second examination 1 year later, thus avoiding seasonal variation. Furthermore, two examinations at this interval fall within the period recommended for calculating the proportion of time that affected persons spend in an episode of arthritis (*8*).

Since the purpose of this presentation is the examination of problems associated with objective measurements, the many additional difficulties peculiar to the physical examination will not be dealt with here. However, a subsample of participants were examined independently by a second physician during the same visit.

For pragmatic reasons, X-rays of the hand were taken only of persons who rated positive on four or more of the possible eight ARA points. As outlined, the effect of omitting the X-ray for the remainder of the population was calibrated by X-raying approximately 20 percent of the population, regardless of their ARA rating. This age-stratified subsample ranged from 15 percent for the younger group to 60 percent for those over 65. These X-rays are being interpreted independently of the clinical data. They are being read in such a way as to measure both intra-observer and inter-observer variability. Serologic tests for the rheumatoid factor were quantitative, rather than qualitative, with full titers obtained for both the bentonite flocculation and the sheep cell agglutination tests. Aliquots of serum from pools of varying titer were repeatedly introduced to measure the technical variation with these procedures. The distribution of the rheumatoid factor and its relation to various grades of rheumatoid arthritis consequently are available. Continuous observation of the patients should give the true worth of the ARA criteria. In addition, the suggested (*15*), although disputed (*16*), value of the titers of rheumatoid factor as predicters of the future development of rheumatoid arthritis can be subjected to critical examination.

Hyperuricemia and gout. With one exception, all the information required to satisfy the ARA criteria for gout was obtained. The exception was the demonstration of urate crystals in synovial fluid or of urate deposition in tissues by chemical or microscopic examination since such procedures did not seem feasible in a community setting. Much preliminary work was done on the uric acid method to validate its accuracy and reproducibility. Using an automated method for the whole population, with the enzymatic uricase method used on a random subsample for reference, will prove invaluable in standardizing the data. In addition, the use of a serum quality control pool with a measured uric acid content allows interpretations in greater depth.

The detailed care given to technical aspects will help to establish confidence in the validity of the resulting data for the distribution of uric acid values and should allow evaluation of currently used norms. The proposed relation to socioeconomic levels will also be available since questionnaire information on the education and occupation of each person was obtained (*17*). Repeat determinations both on the serums of the 5 percent sample recalled for glucose tolerance tests and on the age-stratified sample when reported again 1 year later will allow assessment of the intra-individual variation. This will be of particular value in view of our stringent quality control procedures, which are designed to identify that portion of the variability resulting from technical causes. The clinical significance of hyperuricemia will also emerge as the study continues.

Quality control procedures. In general, serums of known content were included as the first and last samples in each set of determinations. In addition to routine laboratory protocol, the use of these quality control pools, with defined confidence limits, indicated when repeat determinations were to be performed for technical reasons. The following control procedures were also adopted:

1. Measured serum or urine pools, for which all the existing glucose or uric acid was destroyed and varying measured quantities were added to give the pools a range of values from which we could choose. Samples from the different pools were introduced "blind" each day and run with the samples from the study. Large pools were replicated for use throughout continuing phases of the study, allowing quantitation of both accuracy and variability of the methods over time.

2. Duplicates of some specimens were introduced under assumed names, and a random selection of specimens of the same day was reanalyzed at the end of the run. This further assesses the variability within a specific method.

3. The special problems found in gauging the variability of serologic methods were met by using pools of high, low, and borderline titer as guides for the operator. In addition, replicates from pools with varied titers will be fed "blind" to the laboratory throughout the various phases of the study.

4. Five percent of the samples also were analyzed by alternative reference methods (table 3). This increases confidence in the method used for the whole population and facilitates comparisons with other studies.

Since the comparison of one study with another is greatly influenced by the operating level of the laboratory, a unique attempt was made at direct interstudy comparisons. Samples for blood sugar, uric acid, and serologic measurements were mailed to the laboratories servicing three other continuing epidemiologic studies.

Summary

Biological and technical variability have been outlined as sources of often unrecognized variation in the results of epidemiologic studies of chronic disease. Intra-individual fluctuations of biochemical measurements frequently give rise to bias when suspect persons are subjected to confirmatory retesting. This lability, combined with observer variation, also results in cumulative prevalence figures being related directly to the frequency of testing. Epidemiologic studies must quantitate these variations if acceptable prevalence and incidence figures are to be obtained while new information on the natural history of the disease is concurrently documented. Technical error is a more readily recognizable source of variation, but it is often measured inadequately.

The design of a study of diabetes, arthritis, and gout in Sudbury, Mass., is used to illustrate some of the ways that such problems can be handled. These methods consist of (*a*) the use of compensating measures such as the application of diagnostic tests to a preselected random sample of the population in addition to those who screened positive or suspect on initial testing, (*b*) the adoption of quality control procedures allowing measurement of the technical variations with respect to both accuracy and reproducibility over the time period of the study, and (*c*) the retesting of a random sample

Table 3. Laboratory methods adopted for Sudbury study

Determination	Method used	Reference method [1]
Blood glucose	AutoAnalyzer, N-method (*18*)	Somogyi-Nelson (*19*).
Serum uric acid	AutoAnalyzer, N-method (*20*)	Uricase (*21*).
Serum cholesterol	Huang (*22*)	Kendall-Abell (*23*).
Rheumatoid factor	Bentonite flocculation (*24*)	Sheep cell agglutination (*25*).
Urine glucose	Glucose oxidase (*26*)	Froesch-Renold (*27*).

[1] Except for the sheep cell agglutination test, which was applied to the whole population, each reference method was performed on a random subsample.

of persons after a short interval in order to gauge biological variability. Reexamination over longer intervals will provide annual incidence rates. The later development of overt disease will allow validation of initial diagnostic criteria and assessment of the degree to which fluctuating biochemical values affect interpretations.

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Program Notes

Rabies from Wild Animals

More counties in New York State were recorded as having rabies-infected animals in 1965 than in any previous year. Foxes were the chief spreaders of the disease, accounting for 100 cases in the State in 1965. Sixty-six rabid skunks were also reported in New York State in 1965—the highest number in a single year. Rabid skunks are a danger to human beings since they normally live in and near populated areas and rummage in garbage cans or dig for grubs on lawns.

Attack on Mosquitoes

After the 1964 St. Louis encephalitis epidemic which endangered Houston and Harris County, Tex., health authorities launched an all-out attack on mosquitoes.

The county health department printed 600,000 copies of a pamphlet entitled "Stop Raising Mosquitoes in Your Yard and Home" and got them distributed to residents through grocery stores, milkmen, schools, and the Chamber of Commerce.

All incorporated areas in the county were requested to make available all equipment for fogging and spraying their communities and to report weekly on the amount of such work within their areas.

Youth and civic organizations searched out and reported mosquito breeding places. Television and radio stations, civic clubs, and other groups helped complete the initial phase of health education about mosquitoes and disease in Harris County.—*Texas Health Bulletin*

Assessing Air Pollution

The New Jersey State Department of Health is using mobile trailer laboratories to measure and record levels of certain gases and particulates in the ambient atmosphere.

One of the laboratories, at a center-city site in Newark, assesses a heavily populated urban area with a high traffic level. Another mobile laboratory is in Hudson County Park, Jersey City, a site relatively free of such local sources of pollution as automobiles, industrial plants, and power generating facilities. This laboratory affords a reading of pollution of the overall metropolitan air mass. A third trailer on the grounds of the New Jersey State Hospital, Ancora, in southern New Jersey, will measure clean air.

The continuous air monitoring system provides for measurement of sulphur dioxide, nitrogen dioxide, nitric acid, total oxidant, and aldehydes, using a bank of five Technicon AutoAnalyzers.

Suicides in Psychiatric Patients

Fifteen percent of all suicides in Maryland in 1964 were committed by persons currently or previously under care in a psychiatric hospital or outpatient facility. The 59 suicides among the 50,702 adults who had been under treatment in these facilities between July 1, 1961, and June 30, 1964, were equal to a rate of 116.4 per 100,000, compared with a rate of 15.4 for the remainder of Maryland's adult population.—*Statistics Newsletter*, State of Maryland Department of Mental Hygiene, June 10, 1966.

Colorado Air Pollution Act

Enactment of the Air Pollution Control Act by the 1966 Colorado General Assembly has put into operation a plan for ridding Colorado of damage from smog and other air pollution.

The act establishes ambient air standards above which limits of air contamination by particulates and gases are declared unacceptable. It establishes emission (single source) standards which shall be in force in areas designated by the State health department as those in which the ambient air standards are exceeded. Local governments are authorized to enact laws which may not be less restrictive than the State standards, and enforcement shall be the responsibility of a local air pollution control authority or authorities designated by the State health department.

The major air pollution sources which can and will be given attention under the act will be those of emissions from a single stationary source, such as industrial stacks or burning operations, refineries, and burning city or county trash disposal dumps.

Boating and Clean Streams

In Pennsylvania, an estimated 429,000 persons use 110,000 pleasure boats and spend some $37.5 million per year on boating. Pennsylvania's fishermen also spend an estimated $130 million per year for fishing tackle, food, lodging, travel, and related expenses in pursuit of fishing recreation.

Dr. Charles L. Wilbar, State health secretary and chairman of the State's sanitary water board, and other board members have used such estimates to stress the need for assuring "that clean, wholesome waters flow in our streams."

Smallpox Vaccination Law

Governor Nelson A. Rockefeller recently vetoed a bill that the New York State Legislature passed exempting children of members of certain religious denominations from smallpox vaccination requirements.

Present law requires that a child be vaccinated as a prerequisite to attending school in cities having a population of 50,000 or more.

Items for this page: Health departments, health agencies, and others are invited to share their program successes with others by contributing items for brief mention on this page. Flag them for "Program Notes" and address as indicated in masthead.
