tion that is tested has special importance in the interpretation of those diagnostic tests that are the sole definition of a disease.

Such tests are common. Plasma glucose concentration is the sole definition for noninsulin-dependent diabetes mellitus (2), as is plasma potassium concentration for hyper- or hypokalemia, blood pH for acidemia or alkalemia, blood thyroxine concentration for neonatal hypothyroidism, and plasma lipid concentrations for some forms of hyperlipoproteinemia (3).

Diagnostic tests that are the sole definition for a disease cannot be evaluated for sensitivity, specificity, or predictive value, because there are no independent criteria by which to detect the disease. Unfortunately, estimates of sensitivity, specificity, and predictive value for such tests are quite common (1, 4).

Populations are screened for a disease with a test that is the sole definition of the disease in question. Plasma glucose concentration as a test for noninsulin-dependent diabetes is a classical example, although the same scenario applies whenever predictive value theory (1) is used to evaluate a test that is the sole definition of a disease. Subjects exhibiting a plasma glucose concentration exceeding a predetermined value are "screened" as positive for diabetes (4). The test is then repeated with some minor variation such as a stress or tolerance condition. All subjects exhibiting a plasma glucose concentration exceeding a predetermined value in the second test are "diagnosed" as being diabetic. The percentage of the "diagnosed" diabetics that were "screened" as diabetics is accepted as an estimate of the sensitivity of the screening test (4). The percentage of the "diagnosed" nondiabetics that were "screened" as nondiabetics is accepted as an estimate of the specificity of the screening test (4). Thus, the false diagnosis of the original population that was "diagnosed" as being diabetic gives the prevalence of diabetes (4).

From data on test sensitivity and specificity and disease prevalence, it is possible, according to theory (1), to calculate the predictive value of the screening test, and this is what has been reported (4) and accepted as true (1). In fact such an evaluation of the screening test is based on a "diagnostic" test that is synonymous with the screening test and has itself not been evaluated.

The logical fallacy of using a test to define a disease and then using the number of subjects with this definition to evaluate the test has important consequences. If a positive test is the sole definition of a disease and if that test is less than 100% specific, the prevalence of the disease will increase in direct proportion to the prevalence of testing. This can explain the unrealistically high prevalence of diabetes (12%) that has been observed in screening programs (4). According to theory (1), the predictive value of a positive result increases as the prevalence of the disease increases. Thus the test's reputation improves as the prevalence of testing increases. But as a test's reputation for predictive value improves, the tendency to use the test increases, which thereby further increases the prevalence of the disease. The cycle is self-perpetuating and misleading.

Predictive value theory should not be applied to those tests that are the sole definition of a disease.

References

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Obviating Interference with Hydroxyindoleacetic Acid Assay

To the Editor:

We were interested to read that acetic acid, used as a preservative in urine, interferes in Goldenberg's modification of Udenfriend's method for measuring 5-HIAA (1). In this modified method, the same extraction procedure is used as in the original method. We have found that both of these methods suffer interference from acetic acid and have investigated the cause. The extraction procedure involves ether extraction of urine followed by back extraction into neutral buffer. Acetic acid, which is extractable into ether, acidifies the buffer with consequent poor extraction of 5-HIAA. Indeed, in our hands, no 5-HIAA was extracted. We could not overcome this problem by increasing the strength of the buffer. HCl, which did not cause acidification of the buffer, did not interfere with the extraction of 5-HIAA. Methods that avoided the back extraction step were also unaffected by acetic acid. We find these observations surprising as Udenfriend's procedure, which recommends the use of acetic acid as a preservative, is an established method. Indeed, we were following instructions reproduced in a standard textbook (2).

Enzymic Determination of Total Urinary Acidic 3α-Hydroxysteroids

To the Editor:

Recently enzymic methods have been described for determination of neutral steroids such as 3α-hydroxysteroids and 3β-hydroxysteroids (1, 2). The metabolism of acidic steroids has been elucidated by Bradlow et al., using radiolabeled cortisol (3, 4). Here, we describe a method for determination of total acidic 3α-hydroxysteroids by use of 3α-hydroxysteroid dehydrogenase (EC 1.1.1.50). The principle of the method has been described previously (1). The reactions are:

Acidic 3α-hydroxysteroids + NAD+ → 3α-steroids + NADH

5-HIAA + 2-p-iodophenyl-3-p-nitrophenylphosphate → formazan (pink color)

The enzyme color-development reagent was prepared as described (1). All reagents were of analytical grade. 3α-Hydroxysteroid dehydrogenase and β-NAD+ were from Nyegaard & Co., Oslo, Norway. A 1 g/L solution of cholic acid in ethanol was prepared as a standard. The procedure is as follows.

Pipette 10 mL of urine into a 40-mL tube and adjust the pH to 6.5, with bromthymol blue paper as indicator. Add 3 mL of β-glucuronidase (1000 Fishman units/mL), 1 mL of phosphate buffer (pH 6.5, 0.5 mol/L), and a few drops of chloroform, and mix well. Incubate the mixture for 24 h at 37 °C, then adjust to pH 1 with 6 mol/L sulfuric acid and saturate with 5 g of sodium chloride. Shake the solution with 15 mL of ethyl acetate for 5 min. After centri-