hibition produced by the racemic mixture was traced to an isoleucine deficiency resulting from inclusion of *DL*-isoleucine, which was actually a mixture of all 4 isomers, thus effectively diluting the active isomer. Substitution of *L*-isoleucine or a mixture of *L*- and *D* allo-isoleucine for the racemic compound in the *DL* mixture entirely overcame inhibition and produced growth comparable to that obtained with *L*-amino acids. The experiments provide evidence that *D*-amino acids can be effectively used as source of nitrogen for biosynthesis of non-essential amino acids.

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Cholesterol Estimation on Unmeasured Drops of Whole Blood.* (25871)

ANCEL KEYS, MARIO MANCINI† AND JOSEPH T. ANDERSON

Lab of Physiological Hygiene, University of Minnesota School of Public Health, Minneapolis

Risk of future coronary heart disease in middle-aged men is related to concentration of cholesterol in serum measured in apparent health(1,2,3). Accordingly, blood cholesterol measurement is indicated, both in research programs to define relationships more precisely in populations, and in screening and possible preventive programs. Measurement of serum cholesterol is readily made though reports from routine laboratories indicate the need for control(4). But practical requirements limit wide application of cholesterol measurements to scattered populations and insurance applicants. Complications of storing and transporting liquid serum may be avoided by drying a measured amount (0.1 ml) of serum on filter paper, in which cholesterol may accurately be estimated at leisure in another laboratory(5). This requires drawing blood, separating and accurately measuring serum onto paper. These seemingly unexacting requirements are not readily met everywhere and, in fact, impede programs involving cooperation of general practitioners or untrained assistants. To simplify the procedure we studied what may be done with a few drops of finger tip blood, collected on filter paper. The result is a method, adequate for many screening and epidemiological purposes, that makes only trivial demand except at a distant analytical laboratory. Proceeding from the knowledge that drops of serum on filter paper, dried in room air for an hour or 2, may be successfully analyzed for cholesterol months later, trials were made with whole blood; this proved to be stable. The question, then, was to estimate amount of blood used when no measurement is made at time of drawing. Advantage may be taken of the relative constancy in whole blood, except in persons with frank anemia or polycythemia, of concentration of iron, sodium or simply the solids.

Method. Discs of filter paper 4.25 cm di-

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† Present address: Istit. di Patologia Speciale Med. Univ. di Napoli, Italy.

ameter are used (Whatman No. 1 or No. 42 if iron is to be measured). They should be handled with forceps. If whole blood solids are the point of reference, discs are brushed, numbered, dried in vacuum desiccator at least 6 hours, and weighed to 0.1 mg, taking precautions to prevent or correct for moisture absorption after removal from desiccator. We used a micro-torsion balance in dry chamber under slight positive pressure of specially dried nitrogen, manipulation made with rubber gauntlets let into wall of chamber. After weighing, discs are transferred to individual glassine envelopes for mailing or storage. Finger tip is cleaned with alcohol and, when dry, is pricked, allow drops of blood to dribble onto filter paper held with hemostat, then hung by finger loops of hemostat in room air. When paper appears dry (usually 1 to 2 hours) it is returned to glassine envelope and ready for mailing. If either iron or sodium are to be used, weighings are unnecessary. Analysis may be carried out after blood is collected, no refrigeration is required. The paper is removed (forceps) from envelope and again dried and weighed. Cholesterol analysis follows same procedure(5). This involves treatment of paper and blood with alcoholic KOH, extraction with petroleum ether, evaporation of ether extract and development of color with Liebermann-Burchard reagent(6). If iron is to be determined, water and alcohol remaining with residue after petroleum ether extraction are driven off by heat and stream of air. Iron is determined according to method of Snell and Snell(7).

Results. Dried whole blood vs. *serum.* The first series of experiments utilized blood from arm vein from 38 men to provide a wide range of serum cholesterol values. No obvious cases of marked anemia or liver disease were included but the series was not specially screened and included patients with coronary heart disease. Immediately after drawing blood, portions (0.1 ml) were placed on weighed filter paper discs, hematocrit and hemoglobin values were obtained and serum separated for analysis by our version(5) of the method of Abell, *et al.*(6), and also used to measure cholesterol in fresh whole blood. Paper discs were reweighed after drying and, following a few days of storage at ordinary room temperature, were analyzed for cholesterol, as indicated above, and for iron.

TABLE I. Regression Obtained with 38 Blood Samples Covering Ranges 135 to 318 mg Cholesterol/100 ml Serum, Hematocrit Values of 43.6 to 54.1%, and Hemoglobin Values of 13.5 to 17.2 g/100 ml.

	Equation	S.D.
1.	$Hb = 5.81\,Fe + 1.74$	.58
2.	$Vc = 16.1\,Fe + 12.6$	2.04
3.	$W = 21.6\,C + 10.1$	8.00
4.	$Y = 38.9\,C - 84.5$	18.1
5.	$Y = 43.2\,C + 5.04\,Vc - 368$	13.6
6.	$Y = 44.2\,C + 17.3\,Hb - 386$	12.9
7.	$Y = 40.5\,C + 82.8\,Fe - 282$	16.2
8.	$Y = 71.1\left(\frac{C}{Fe}\right) - 24.1$	19.5
9.	$Y_{1,2} = Y_{1,1}$	5 *

* Stand. error of measurement.

Abbreviations: Y = cholesterol, mg/100 ml fresh serum. W = cholesterol, mg/100 ml whole blood. C = cholesterol, mg/mg dried whole blood solid. Fe = iron, mg/mg dried whole blood solid. Hb = hemoglobin, g/100 ml fresh whole blood. Vc = hematocrit, ml/100 ml fresh whole blood. S.D. = stand. dev. from regression, same units as of predicted item (left hand of equation).

Table I summarizes results in terms of regression equations obtained by least squares and standard deviations from regression. Results show that concentration of cholesterol in mg/100 ml of fresh serum can be estimated from cholesterol/unit weight of dried whole blood solid by means of equation No. 4, standard deviation from regression being 18.1 mg/100 ml, or 7.6% of mean of 238.8 mg/100 ml in these 38 samples. As expected, hemoglobin, hematocrit and iron weight/unit weight of dried whole blood are closely related. Since cholesterol concentration/unit of cell volume is remarkably constant(8), inclusion of some measure of cell volume in an estimating equation should improve estimation by correcting for variability in hematocrit. S. D. values associated with multiple regression equations No. 5, 6, and 7 show this, though improvement gained by adding Fe data (with S.D. = 16.2) is not great as compared with prediction omitting this (S.D. = 18.1).

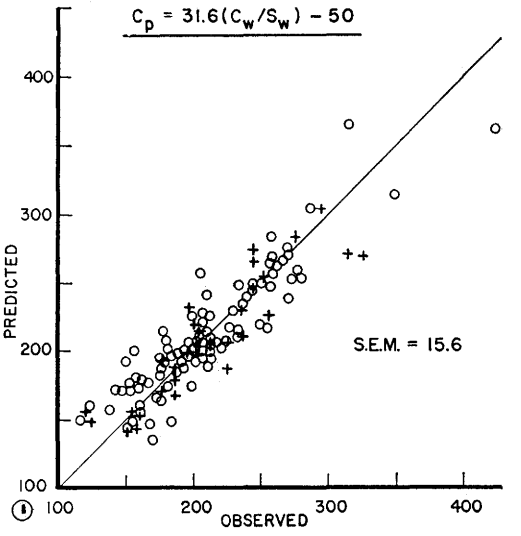
Equation No. 8 is least squares regression

equation for estimation of serum cholesterol from ratio of cholesterol to iron in dried whole blood. The standard deviation of 19.5 mg cholesterol/100 ml of serum for the difference between observed and predicted values is larger than in estimation from cholesterol unit of whole blood solid (equation No. 4) and suggests that iron as sole reference point is less satisfactory than blood solids.

Results with equations 4 through 8 may be compared with equation 9 which indicates average standard error of measurement when duplicate analyses are made with the standard method of aliquots of the same serum. But, as shown below, the standard error of measurement in equation No. 9 gives a false impression of reliability of single serum cholesterol measurement in estimation of the true mean value of the individual.

Estimation from finger tip blood. The foregoing refers to blood drawn from arm vein by syringe. A new series of 93 men was studied to discover relationship between cholesterol concentration in arm vein blood serum and that per unit weight of whole blood solids dried on filter paper, using first 3 to 5 drops of finger tip blood. As before, men included coronary patients as well as men ostensibly healthy or with only minor complaints; ages ranged from 20 to 65, mean = 53 years. Directly measured serum values ranged from 117 to 423 mg/100 ml, mean = 210.6, and these were obtained independently by different technicians than those who worked with dried whole blood.

The coefficient of correlation between cholesterol concentration directly measured in serum (mg/100 ml) and that measured in dried spots (mg/g of solid) was r = 0.892 in this series and the regression equation was obtained by least squares: 10. $\tilde{Y} = 31.6\ X - 50$ where $\tilde{Y}$ is predicted cholesterol in mg/100 ml of serum and X is that in spots, mg/g of solids. Standard error of measurement, considering observed and predicted values of serum cholesterol as 2 measurements of the same item and computing S.E.M. as usual in that situation ($S.E.M.^2 = \Sigma\Delta^2/2N$), gave S.E.M. = ± 15.6 mg/100 ml.



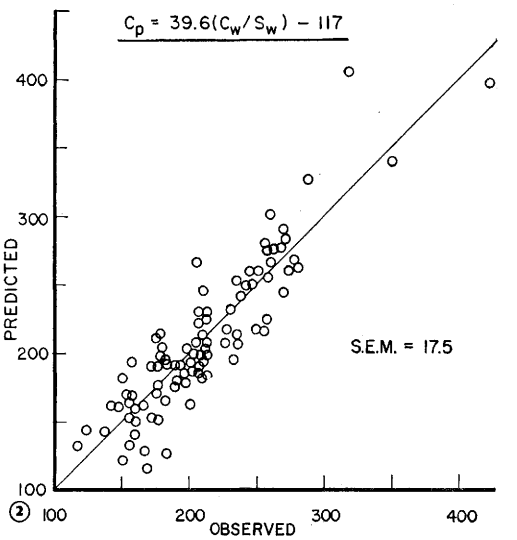


FIG. 1. Observed serum cholesterol measured in arm vein blood plotted against value predicted from ratio of cholesterol to whole blood solids, using the least squares equation (No. 10). ○ = 93 samples used in derivation of equation No. 10. + = subsequent trials with other men.

FIG. 2. Same as Fig. 1 but predicted values were obtained by use of the theoretical equation No. 11a.

Fig. 1 shows serum cholesterol values observed and predicted from equation 10 in this series of 93 samples plus 31 samples analyzed later and not included in derivation of equation No. 10.

Prediction equation from theory. A theoretical equation for prediction of serum cholesterol from cholesterol/unit weight of dried

whole blood can be derived: Let C be concentration of cholesterol in mg/100 ml, with subscripts p, c, and w representing serum (or plasma), cells, and whole blood respectively. Let S be weight of solids in g/100 ml, with subscripts as above. Now, if H is hematocrit, ml of cells/ml of whole blood, we have: $C_w = HC_c + (1\text{–}H)C_p$, and $S_w = HS_c + (1\text{–}H)S_p$. Accordingly, concentration of cholesterol in mg/g of whole blood solids will be: $\frac{C_w}{S_w} = \frac{HC_c + (1-H)C_p}{HS_c + (1-H)S_p}$, from which concentration of cholesterol in mg/100 ml of serum is, 11. $C_p = \frac{C_w}{S_w}\left(\frac{HS_c}{1-H} + S_p\right) - \frac{HC_c}{1-H}$. This corresponds to regression equation: $C_p = a + b\frac{C_w}{S_w}$, where $a = -(HC_c)/(1\text{–}H)$ and $b = (HS_c)/(1\text{–}H) + S_p$. We found $C_c = 137$ mg/100 ml with great constancy in human blood(8) so coefficient a may be computed for various values of the hematocrit, H. Computation of coefficient b requires estimates of concentration of solids in serum (S_p) and in cells (S_c). Ranges of these variables to cover 95% of bloods of adults are (9): $S_p = 7.9$ to 9.1, $S_c = 34$ to 39. Mid-points of these ranges, 8.5 and 36.5, respectively, correspond closely to our own findings for medians for adult men aged 20 to 65. Using these values, coefficient b may also be computed for various levels of hematocrit.

In our series of 93 samples to obtain equation No. 10 the hematocrits are not known but mean value may be estimated from equation 11 since we have directly measured mean $C_p = 210.6$ from which $H = 0.46$ ml cells/1 ml of whole blood and the numerical solution of equation No. 12 is: 11a. $C_p = 39.6\ C_w/S_w - 117$. When equation 11a is applied to the data from the 93 samples the difference between means of observed and estimated serum cholesterol values is 0.26 mg/100 ml, and S.E.M. ± 17.5. Observed and predicted values are shown in Fig. 2. This theoretical estimating equation is almost as good for prediction as the empirical least squares solution, in spite of superficial difference between coefficients.

TABLE II. Repeatability and Validity of Estimated Serum Cholesterol from Cholesterol per Unit Weight of Whole Blood Solids. All values in mg of cholesterol per 100 ml of serum.

$(S.E.M.)^2 = \Sigma\triangle^2/2N$.

Directly measured serum, mean	199.5
Estimated from whole blood, sample 1	197.3
Idem " 2	200.2
S.E.M. between samples of whole blood	±10.8
" " direct and whole blood estimate	±15.3

Repeated finger tip punctures. It is not suitable to take duplicate samples from the same finger prick unless an undesirably deep puncture is made. After taking first few drops as sample No. 1. a second sample requires "milking" the finger with resulting increased variability and lower average cholesterol concentration/unit of blood solids, presumably because of dilution with tissue fluid. Accordingly, comparisons were made between findings on bloods from 2 separate finger tip punctures on each of 13 men. An arm vein sample was obtained at about the same time. Data are summarized in Table II, serum cholesterol estimates from finger tip bloods being made by equation No. 10. Standard error of measurement, direct serum cholesterol *vs.* estimate from cholesterol/unit whole blood solids was ± 15.3 mg/100 ml. This agrees with corresponding value for the 93 sets of data used to obtain equation 10 (S.E.M. = ±15.6). S.E.M. for serum cholesterol values estimated from dried whole blood sample 1 *vs.* 2 was ±10.8 mg/100 ml and represents method error plus biological variation between bloods from different fingers punctured 10 minutes apart.

Discussion. Theoretically, estimation of serum cholesterol concentration from ratio of cholesterol to sodium in dried whole blood may be better than with whole blood as reference. The 95% range in normal man is only from 132 to 144 meq of sodium per liter of plasma, the average for blood cells is about 18 meq per liter of cells(9). Work on the method with sodium is in progress.

In screening and in epidemiological studies a major purpose for estimating cholesterol in

TABLE III. Intra-individual Variation in Serum Cholesterol Concentration, mg/100 ml. "No. pairs" = number of men except in series 2 where a total of 110 men provided 834 pairs of observations, each 1 to 2 weeks apart, over period of 3 years.
$(S.E.M.)^2 = \Sigma\triangle^2/2N$, where N = No. of pairs.

Series	Interval	Diet, etc.	Age	No. pairs	Chol., mg/100 ml Mean	S.E.M.
1	1-2 wk	Fixed	20-29	38	209.7	12.0
2	"		35-60	834	228.2	11.5
3	1 yr	*ad lib*	20-29	100	175.8	18.2
4	"		55-64	100	234.9	20.1

the blood is its possible use to identify risk of future coronary heart disease. In the Framingham Study of U.S. Public Health Service a cutting point of 260 mg of cholesterol per 100 ml was used(2). From a follow-up of 4 years it was reported that men with single sample values above 260 subsequently suffered 2.9 times the rate of new coronary disease experienced by men with cholesterol values under 260. In the Albany Study of N. Y. State Dept. of Health(3) a cutting point of 275 mg/100 ml was used. Men above that level had a coronary attack rate 3.4 times greater than men below that level. Both Albany and the Framingham data provide valid estimates of prognostic significance of single cholesterol measurements but they must be gross under-estimates of significance of true mean values. This follows from a consideration of intra-individual variability.

Table III summarizes data on intra-individual variability in serum cholesterol. Series 1 and 2(10) refer to samplings 1 to 2 weeks apart from men maintained throughout in a constant state with regard to amount and composition of diet, exercise, recreation and environment. Series 3 and 4 refer to samplings just one year apart (same season), from men of settled habits and occupation in same state of health (clinically healthy), and metabolic state (resting, fasting), on 2 occasions. Values in Series 3 and 4 must be under-estimates of average intra-individual variability in ordinary life over a period of one year.

For men living ordinary lives intra-individual variability over any appreciable period of time, in the directly measured serum cholesterol, is larger than same-day variability between estimate from finger tip whole blood and measured arm vein serum cholesterol. Besides suggesting that the dried blood estimate is relatively good, these data are important in evaluating such material as from Framingham and Albany. Since single blood samples are imperfect indicators of true mean cholesterol values of individuals, any classification of men above and below any cutting point on the basis of single blood samples will misclassify some men in regard to their true mean values. From distribution of individual (single sample) values in a group, and data as in Table III, frequency of misclassification above and below any selected cutting point may be estimated.

Elsewhere mathematical theory and application will be presented. Here it is enough to state that such analysis of Framingham and Albany data indicates that true individual mean values of serum cholesterol must show some 60 to 80% better separation of high risk from low risk men than reported.

Considerations of intra-individual variability indicate: 1) Real significance of serum cholesterol level as predictor of risk of coronary heart disease has been under-estimated in analyses reported heretofore. 2) Much improvement in reliability for prognosis of cholesterol measurements will be gained from a series of bloods taken from each individual at different times.

Summary. A method is presented for estimation of serum total cholesterol concentration from concentration of cholesterol/unit of whole blood solids, using a few unmeasured drops of finger tip blood dried in room air on filter paper. Prolonged storage at ordinary temperatures may elapse before analysis. In 124 sets of comparisons between se-

rum from arm vein and dried finger tip blood, standard error of measurement was ± 15.5 mg cholesterol/100 ml serum. This is comparable to intra-individual variability in direct serum measurement of blood samples drawn a few days apart and is smaller than variability between serum values in casual bloods drawn at longer intervals. Significance of blood cholesterol level in prediction of risk from coronary heart disease has been under-estimated in previous studies.

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Erythropoietic Recovery Measured by Fe_{59} Uptake in Irradiated Mice Protected with Bone Marrow.* (25872)

E. A. MIRAND, J. G. HOFFMAN AND T. C. PRENTICE

Roswell Park Memorial Inst., and Physics Dept., University of Buffalo, N. Y.

Lethal effect of whole body irradiation of mice and other mammals(1,2) is offset by intravenous grafting of nonirradiated bone marrow which may be isologous, homologous, or heterologous. It is believed that the introduced marrow cells proliferate and provide recovery by cellular repopulation or by elaborating some humoral substance which stimulates recovery of indigenous marrow. It is possible too, that transfused marrow plays a beneficial, transitory function pending recovery of indigenous marrow. Survival of animal after lethal doses of radiation depends on time of hemopoietic recovery. This has been measured in mice by recovery of the count of circulating lymphocytes and granulocytes and by recovery of hemoglobin concentration and platelet count. Recovery of hemoglobin concentration is not a satisfactory index of recovery because it is obscured by the slow downward trend of circulating survivor cells over a period of 12 to 16 days after radiation (4). Odell and Caldwell(5) found donor type erythrocytes in irradiated rats in significant numbers only after 14 to 20 days. To examine quickly the recovery of erythropoiesis we used the Fe_{59} uptake method(6) and find that recovery begins after third day post-radiation and is discernible by sixth day when isologous cells are administered. Moreover, responses elicited by homologous and heterologous cells are measurably different from one another and from the isologous case by sixth day. The method appears to distinguish the 3 genetically different cell types by the induced Fe_{59} uptake.

Methods and materials. ICR/Ha Swiss male mice, 6 weeks old weighing 20-25 g were irradiated at target distance of 30 cm, 250 kv, filter ¼ mm Cu plus 1 mm Al, with a dose of 900 R. Survival was 50% at 6 days and 0% at 12 days. Protection was given with isologous bone marrow from 6-week-old cou-

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