

The observation of the development of the sensitivity of the operator in the course of the experiments are summarized in figure 7. Such observations had been made many times with different operators. They normally were a 'by-product' of planned investigations.

To exclude an unconscious guess of the source position, we performed some blank experiments. During these the operator was on the trolley without his divining rod and the source was inactive. Before putting on the blindfold, the operator was shown an arbitrarily chosen location he had to remember. He then tried to guess the moment he was passing the imagined location on the moving trolley, which he would indicate by rising a hand. Such blank experiments were made with 4 operators. Figure 8 shows 2 diagrams obtained with operator ER: on top a real experiment with the rod from April 17, 1975. This experiment equally shows the 'tuning-in' of the operator who performed some 15 min later the excellent series given by figure 5, ⊙. At the bottom, a blank experiment from June 11, 1975. The absence of guesses – the operator declared complete loss of orientation – did not permit to evaluate numerical values.

Such blank experiments with operators on foot revealed for some operators a remarkably precise guess. Therefore only trolley test experiments for locating of an unknown source position were accepted.

Why did we not change the position of the source under the platform? Initially it was intended to do so, permitting the operator to work without any hinderances such as blindfolds or trolley travelling. We observed that when the operator initially located his reactions at A, in or near the perpendicular of a given source position, then, after changing the latter in a direction and amount unknown to the operator, he normally first detected the old reaction place A which then gradually shifted to a new place A' within 5–15 min, the difference A–A' reflecting the displacement of the source in direction and amount. This phenomenon was repeatedly observed with different operators. From this observation it follows which sort of difficulties we encountered in realizing the very first program step with the platform facility and the artificial source.

Conclusions. Do the described experiments answer the question of the perceptibility of the artificial source? The experiments of the type 'water running or not' can be regarded as statistically confirmed proofs. The risk of being wrong in rejecting the pure random hypothesis with $p = 1/2$ is very small.

The experiments of locating the source position have to be divided in 2 categories: a) Variable source position; operator working without constraint. In many series the average locations of the reactions corresponded within the statistical fluctuations with the vertical of the source position. Moreover many other observations revealed systematic deviations of the average location which, nevertheless, was seemingly correlated with the source, for a displacement of the latter reflected in a progressive, finally equal shift of the average location of the reactions; retardations of up to 15 min have been observed. The behaviour of a hypothetical D-field around our surface source might indeed considerably differ from such a field of a deeply buried (natural) source.

b) Fixed source position; operator blindfolded on trolley. These series intended a closer approximation to natural conditions of the source (stable in space and time). The results from blindfolded, trolley-travelling operators were compared to pure guessings under similar conditions but without rod and with inactive source, thus discriminating between a D-detecting capability and an orientation capability. The perceptibility of the source and detection of its environment was documented by reproducible reactions in the vicinity of the vertical of the source, the statistical fluctuations of the reaction locations being small compared to those of the guessing series.

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- 2 A. M. Comunetti, *Experientia* 34, 889 (1978).
- 3 Y. Rocard, *Le signal du sourcier*. Dunod, Paris 1962.
- 4 A. Drigo, *Relazione tenuta al XIV convengo della salute*, Ferrara, 27.5.1967.
- 5 H. L. Koenig, *Unsichtbare Umwelt*. Heinz Moos Verlag, München 1975.
- 6 Z. V. Harvalik, *Virg. J. Sci.* 21, 59 (1970).
- 7 Z. V. Harvalik and W. de Boer, *The American Dowser* 14, 4 (1974).
- 8 W. A. Tiller, 'Devices for Monitoring Non Physical', in: *Psychic Exploration*, Ed. E. D. Mitchell and J. White. Putnam Press, New York 1974.
- 9 Abbé Mermet, *Comment j'opère*. Jussy (Geneva) 1935.

PRO EXPERIMENTIS

A new simple assay for total blood lipids by refractometry

B. Payne, D. J. Galton and D. J. Moul

Lipid Research Laboratory, St. Bartholomew's Hospital, London EC1 (England), 13 June 1978

Summary. A fast simple method for assay of total blood lipids has been devised by precipitating blood proteins with methanol and extracting lipids into diethyl ether. The change in refractive index of the methanol-ether due to dissolved lipids is measured in a refractometer.

The hyperlipaemias are a common group of disorders occurring in the general male population of the United Kingdom at a frequency of about 16% (hypertriglyceridaemia 14%, hypercholesterolaemia 2%)¹. The importance of the hyperlipidaemias is based on epidemiological studies

showing them to act, like hypertension and diabetes, as major risk factors for the development of coronary and peripheral arterial disease²⁻⁴. Because of the high incidence of hyperlipaemia in the general population a simple screening procedure suitable

for hospital clinics and community screening programmes would be of value to identify individuals with raised blood lipids.

We have therefore devised a fast, inexpensive assay for total blood lipids which will indicate which patients require further lipid analysis. The principle of our assay is to extract blood lipids into methanol-ether (1:2 v/v) and then to measure the change in refractive index of the apolar phase containing the dissolved lipids in a portable or bench refractometer.

Methods. Blood was collected from a random sample of patients attending St. Bartholomew's Hospital and the serum prepared for routine lipid analysis. Serum cholesterol was measured by an automated modification of the Liebermann-Burchard reaction and triglyceride by a modification of the Hantzsch reaction⁵. A further aliquot of serum was extracted with an equal volume of methanol-ether (1:2 v/v). The serum proteins are denatured by the methanol and the lipid is extracted into the ether phase. The refractive index of the ether phase is measured in a portable refractometer (Atago 320) sensitive to changes of 0.1 g/100 ml of dissolved solids or in a bench refractometer (Abbe model 60). The time of the assay for lipid extraction is approximately 2 min and the refractive index of a sample can be measured in 30 sec.

Results. The coefficient of the variation of this method for 10 determinations of a single lipid extract is 4.3% and the recovery of lipids after the first ether extraction from serum is greater than 95%. The method using a portable refractometer is sensitive to changes of approximately 0.1 g/100 ml of dissolved lipid (figure 1) and is linear in the normal range of total blood lipids (0.7–1.2 g/100 ml) up to concentrations of about 5 g/100 ml. The method can distinguish between the upper and lower limits of a normal range of serum lipids (i.e. 0.9–0.3 g/100 ml, $n=25$, $p<0.001$) and can easily detect high values. Greater precision for the measurement of individual samples can be obtained with a bench refractometer. In a consecutive series of 50 sera the correlation coefficient between total lipids measured by autoanalyzer and by bench refractometer is 0.89, $p<0.001$ (figure 2). If whole blood is extracted instead of serum some sensitivity is lost because approximately 50% of total lipids are from cell membranes. However measurement of a consecutive series of 65 extracted blood samples by refractometry and serum lipids by the autoanalyzer gave a correlation coefficient of 0.77 ($p<0.001$).

Discussion. This simple method clearly has potential for the identification of individuals with raised blood lipids in community screening programmes. No reagents are required for the assay, which is rapid and easy to perform. Although it is only semiquantitative, it indicates which subjects may require further lipid analysis.

The major limitations of the assay are: the effect on the refractive index of light is a nonspecific property of molecules and cannot be used to distinguish between different lipid classes such as cholesterol or triglyceride; and other blood lipids not measured by routine autoanalyzer methods (such as red cell lipids and phospholipids) will interfere and may be expected to contribute about 50% of the total extractable lipids measured in the refractometer⁶. However, phospholipids are an integral part of lipoproteins and increase in parallel with increases in either low or very-low density lipoprotein classes. If the haematocrit value varies from 45 to 55% this would only cause a 5% error in measurement of total blood lipids (assuming that red cells contribute about one half of total blood lipids)⁶. Other

blood lipids that may also interfere with the assay are bile acids, carotenoids and possibly unconjugated bilirubin⁷. However, these are usually at much lower concentrations than the structural blood lipids and in usual circumstances will not interfere. Another limitation of the method arises from the design of most refractometers for use with aqueous solvents, and an inaccuracy arises with lipid solvents which tend to evaporate rapidly. However with careful use quantitative results can be obtained with the method and it should provide a useful means of screening individuals for hyperlipaemia.

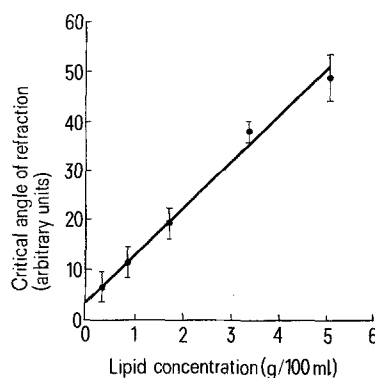


Figure 1. A standard curve of lipid concentration (g/100 ml) versus the critical angle of refraction. The change in refractive index of methanol-ether is plotted against increasing concentrations of lipid (as triolein). Points are means of at least 8 measurements with $2 \times$ SD enclosed between the bars. The normal range of total serum lipids is between 0.3 and 0.9 g/100 ml.

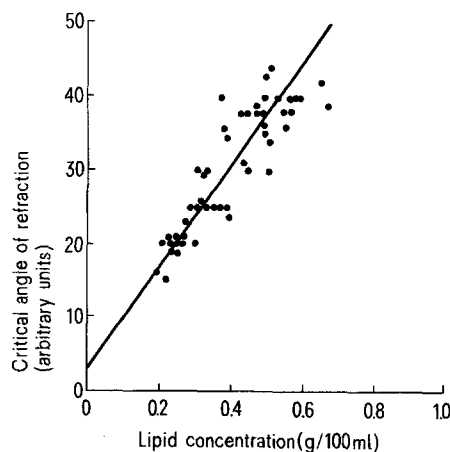


Figure 2. Angle of refraction measured by the bench refractometer and total serum lipids measured by the autoanalyzer. Each point is an individual serum sample measured in duplicate. The correlation coefficient (r) = 0.89, $n = 50$, $p < 0.001$.

- 1 B. Lewis, A. Chait, I.D.P. Wootton, C.M. Oakley, D.M. Krikler, G. Sigurdsson, A. February, B. Maurer and J. Birkhead, *Lancet* **1**, 141 (1974).
- 2 M. Albrink and J. Meigs, *Ann. N.Y. Acad. Sci.* **131**, 673 (1965).
- 3 T. Gordon, W.P. Castelli, M.C. Hjortland, W.B. Kannel and T.R. Dawber, *Am. J. Med.* **62**, 702 (1977).
- 4 J.L. Goldstein, W.R. Hazzard, H.G. Schrott, E.L. Bierman and A.G. Motulsky, *J. clin. Invest.* **52**, 1533 (1973).
- 5 M.J.P. Higgins and D.J. Galton, *Eur. J. Clin. Invest.* **7**, 301 (1977).
- 6 Documenta Geigy, p. 600 (1970).
- 7 W. Sunderman, *J. biol. Chem* **153**, 139 (1944).