Automatic determination of blood cholesterol

J. BOY  From the Civil Hospital, Rheims, France

Manual methods for measuring cholesterol are numerous, some simple, others complicated, and there is as yet no common agreement as to the value of all these techniques. The many causes of error have already been studied (Fürst and Lange, 1954; Rivin, Yoshino, Shickman, and Schjeide, 1958). Even in highly complicated methods, errors may arise at each stage of the analysis if the conditions are not identical, especially in unskilled hands. A method which gives excellent results when a small number of samples is being run will be faulty when applied to the determination of a series, because the conditions of time and temperature are not rigorously the same at the beginning and at the end of the series. It seems, therefore, that an automatic method might offer the means of a more exact determination of a large number of samples. Moreover, the automatic method permits the evaluation of the reproducibility of the reaction. So far it has been possible to transfer only simple manual methods to the automatic level.

METHOD

The reaction used is that of Pearson, Stern, and McGavack (1953) with the proportions of the reagents slightly modified (Boy, Bonnafé, and Mazet, 1960).

REAGENTS  Two reagents are required, the first a mixture of acetic anhydrid, paratoluene sulphonic acid, and acetic acid. In 600 ml. of acetic anhydrid dissolve, without heating, 30 g. of paratoluene sulphonic acid (this is dangerous only when heated in a direct flame). To dissolve the mixture in the acetic anhydrid is easy but it may be slightly cloudy with certain qualities of paratoluene sulphonic acid; this is of no importance. Complete to I litre with glacial acetic acid (400 ml.). The mixture does not develop too much heat with glacial acetic acid of good quality.

The second reagent is pure sulphuric acid (R.G.).

APPARATUS  The apparatus consists of one sampler, one proportionating pump, several jacket coils 16 cm. long, one photometer, and one recorder1.

The kind of tubes used for pumping is important. The sulphuric acid should be pumped with a fluoror tube2 which alone will resist its corrosive action, although it will not withstand the mixture of acetic anhydrid, paratoluene sulphonic acid, and acetic acid. For this reagent siliconized tubing must be used which is resistant if the reacting agents are not allowed to stand in the tube, which should be rinsed after using, and then immersed in water for several hours. The samples are placed on the sampler in alternate cups, leaving every other cup empty. Figure 1 illustrates the arrangement of the tubes.

PROCEDURE  A stream of reagent 1 is pumped at the rate of 12 ml. a minute, then, after having been heated to 65°C. in jacket coil A, is mixed with a stream of serum flowing at 0-3 ml. a minute. The mixture is heated and mixed in a second jacket coil (B) at 65°C. It is then cooled by passing through a third jacket (D) outside which ordinary tap water is running at 15°C. The sulphuric acid is mixed at the rate of 0-6 ml. a minute and takes place in an ordinary coil, the fluid then passing through a final jacket coil (C) in which water is circulating at 65°C. The colour develops and is read with a photometer and recorded.

The standards for the reaction are made with Versatol3. Since the reaction strictly follows the Beer-Lambert law, higher values are easily calculated by multiplying the optical density by 2, 3, 4, so that the absorptions correspond to 2, 3, and 4 times the value of the Versatol.

The extraction of the cholesterol by means of a solvent of the user's choice, followed by the recovery of the extract by a volume of glacial acetic acid equal to the quantity of the sample, permits the analysis of strongly icteric sera and of haemolytic sera.

DISCUSSION

The automatic determination of cholesterol (which we have been practising for three years) has shown the importance of factors insufficiently regarded in the manual methods.

Pearson's method presents the advantage of being carried out on pure serum without extraction, which eliminates from the start the numerous errors caused by extraction in work on a series. A slight modification of the original technique permits of a clear mixture of serum and acetic anhydrid, which is an essential condition for an automatic method. Moreover, the presence of the paratoluene sulphonic acid stabilizes the colour of

Received for publication 18 June 1962

1 All obtainable from Technicon International Ltd. (Autotechnicon), Hanworth Lane, Chertsey, Surrey.

2Versatol: Warner-Chilcott

the Liebermann's reaction, which is important. Furthermore, Liebermann's reaction has good specificity. Only certain sterols included in the insaponifiable can give it. Salkowski's coloration, used in Zak's technique, can be developed by other compounds than sterols and accidental impurities in reagents can produce it.

The three advantages of Pearson's reaction are limpidity of the mixture, specificity, and greater stability. Pearson's method, however, has been criticized for giving too high results, which are attributed to the colour of the serum or to a 'protein effect'. This does not seem to be true if the precaution is taken of cooling the mixture of serum and acetic anhydrid before adding the sulphuric acid; with the automatic method it is easy to cool the samples identically before adding the sulphuric acid. The excess of coloration noted in the manual method is due to the browning of proteins by the sulphuric acid. This does not happen when the mixture is well cooled beforehand.

The second criticism is that the method is not applicable in cases of haemolysis or jaundice. Haemolytic and icteric sera form a minority of the samples for analysis. It is always possible to avoid errors for these few samples either by taking extractions or by making a correction,
Technical methods

which, when the automatic apparatus is used, is valid.

A more serious difficulty is that of standardization
and is not peculiar to Pearson's method; it is solved by
the use of a commercial standard serum of the Versatol
type. Only comparison with a standard serum of good
quality is valid (Rivin et al., 1958), for artificial titrated
solutions never have exactly the same degree of humidity
as the serum and the slightest difference in the quantity of
water causes enormous differences in the intensity of
Liebermann's or Salkowski's reactions.

Since the time of the circuit is rigorously constant, it
has been possible to study the influence of continual
variations in temperature (Fig. 2). A temperature of
65°C. appears best because between 56°C. and 72°C.
variations have very little effect on the development of
the colour; thus even a small variation in the thermostat
does not produce appreciable errors (Fig. 3). Furthermore,
at this temperature the levels of the colour for free
cholesterol and for esterified cholesterol are identical
(Girard and Assous, 1962).

SUMMARY

The use of an automatic apparatus with a strictly con-
tant time for the reaction and the mixing processes
eliminates the errors observed in the manual methods.

An installation is proposed in which the temperature
is fixed in such a way that reproducibility is good such
as was never achieved manually with series including
many samples.

REFERENCES

335.
25, 813.
J. Amer. med. Ass., 166, 2108.

Separation of human lymphocytes
and monocytes using an 'oil bottle'1

IRA GREEN and WILMA SOLOMON From the
Haematology Department, Laboratory Division,
Montefiore Hospital, New York, N.Y.

Several methods of separating lymphocytes and mono-
cytes based on specific gravity differences between
lymphocytes and other types of cells have been described
(Kline, 1955; Ottesen, 1954; Seal, 1959; Tullis, 1952;
Ventske, Perry, and Crepaldi, 1959). The viability of
the isolated cells has usually been tested by indirect methods
(Jago, 1956; Lalezari, 1962; Lapin, Horonick, and
Lapin, 1958). The technique to be described is simple,
requiring only one centrifugation; it is reliable, and uses
equipment which can be readily purchased. No haemo-
lytic agents are used to eliminate the red blood cells. The
viability of the cells obtained using this technique has
been directly assayed by the ability of these cells to
proliferate in subcutaneously placed micro-diffusion
chambers in human subjects.

METHOD

All glass surfaces were siliconized and all equipment and
reagents were sterilized. Sixty to 80 ml. of blood was
collected with a plastic tube2 and led directly into a
100 ml. graduated flask containing 20 ml. of 6% dextran
in isotonic saline (M.W. 188,000)3 and 1 ml. of 10% E.D.T.A. The flask was stoppered and inverted gently
eight times. The erythrocytes were allowed to settle by
gravity for 20 minutes at room temperature. The super-
natant plasma was placed in a 100 ml. 'oil bottle' with a
0-5 ml. stem 4 cm. long, with a diameter of 3-8 mm.
(Corning Glass Co., Corning, N.Y.). The 'oil bottle' was
then spun at 2,500 r.p.m. for 30 minutes in a no. 2 in-
national centrifuge. To hold the 'oil bottle' special cups
(international no. 395) were used.

All the cellular elements of the plasma were now
layered in the narrow dependent stem of the 'oil bottle'
with the platelets uppermost, followed by the lymphocyte-
monocyte layer, then the polymorphonuclear cell layer,
and finally a button of red cells at the bottom. The line of
demarcation between these layers was easily seen (Fig. 1). The supernatant plasma was poured off. The
thick platelet layer was removed using a Pasteur dispos-
able pipette4 and a rubber bulb.

To remove the lymphocyte-monocyte layer a narrow
glass pipette 10 cm. long was fashioned from the long
stem of a disposable pipette. The tip of this pipette should

1Supported by United States Public Health grant no. C-4788.
2Blood Collection Set no. 36, Abbott Labs., Chicago, Ill.
3Pharmachem Corp., Bethlehem, Pa.
4Transpet, Clay-Adams, New York City

Received for publication 25 June 1962
Automatic determination of blood cholesterol

J. Boy

*J Clin Pathol* 1963 16: 178-180
doi: 10.1136/jcp.16.2.178

Updated information and services can be found at:
http://jcp.bmj.com/content/16/2/178.citation

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/