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All moneys should be paid to the Treasurer of the New Zealand Association of Bacteriologists (Inc.), Mr J. P. Walsh, Pathology Department, Green Lane Hospital, Auckland.

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THE CONFERENCE COMMITTEE.
SERUM CHOLESTEROL
A CONSIDERATION OF A LARGE SERIES OF FREE, TOTAL AND ESTER CHOLESTEROL ESTIMATIONS
By IAN D. SCOTT and C. S. O. SHEPHERD
(Thames Hospital)

Cholesterol is the principal member of the large variety of sterols present in the animal organism. It is present in all cells of the body in greater or lesser extent, but is found in its greatest concentration in the liver and the brain. Most of the cholesterol is found in the form of esters of fatty acids, but a variable proportion is in the free form.

Under ordinary circumstances the cholesterol in the serum is measured as the total cholesterol only, no attempt being made to estimate the ratio of free to ester cholesterol. We have found on innumerable occasions that whilst the total cholesterol is within normal limits there is gross diversion from the normal ratio of free to ester cholesterol, especially in such conditions as infective hepatitis in the acute stage, therefore we have made it a practice to estimate the fractions in each routine estimation, and it is the results of a large series of such tests which have prompted this paper.

MATERIAL AND METHODS
Material from which the data in this paper was obtained was in the main, sera which were submitted for cholesterol estimation to assist in the diagnosis of the various thyroid states. A number of estimations were made on sera from proved cases of infective hepatitis and obstructive jaundice as we were interested in assessing the value of the estimation of the ratio of free to ester cholesterol in these cases as an additional test of the liver function.

The sera were kept in the refrigerator until such time as was convenient, and the analyses were made in batches of four to six tests. In all 274 sera were tested over a period of about fifteen months, and the detailed results of this series will be presented later in this paper.

The analyses were performed by a modification of the classical method of Schoenheimer and Sperry which involves the precipitation of the free cholesterol as the digitonide, and the saponification of the total cholesterol and subsequent precipitation as digitonide. The colour is developed by the usual Leibermann-Burchard reaction.

At the same time the majority of the specimens were also
analysed for total cholesterol by the King modification of the Sackett method which is a "direct" method, also employing the Leibermann-Burchard reaction. The results of these duplicate tests will also be presented later.

A few sera were also tested by two recently published methods employing acidic ferric chloride as the colour reagent. These methods show distinct promise and if they prove reliable they will be a great advance on the present methods available for the estimation of cholesterol as they are not dependent on the unstable Leibermann-Burchard reaction which is unsatisfactory for a variety of reasons.

**CHOLESTEROL METABOLISM**

In order to be able to assess the results of an analysis of the serum for cholesterol it is necessary to have some knowledge of the role cholesterol plays in the body metabolism. It is therefore our intention to give a brief outline of these mechanisms with that end in view.

As is well known, cholesterol is one of the group of lipids which form a considerable proportion of the total serum solids, and include such substances as the fatty acids, phospholipids, and the neutral fats. Chemically, it belongs to the sterol group which is widely distributed in nature. In common with other members of the group, cholesterol has the cyclopentanoperhydrophenanthrene nucleus. It differs from other members of the group in the configuration at C17, and by means of a carboxyl group at C3, combines with fatty acids to form esters. It evidently shares with the phosphatides the property of serving as a vehicle for the fatty acids, although the fatty acids in cholesterol tend to be more unsaturated than those in the phosphatides. The concentrations of cholesterol and phospholipids in the blood parallel one another, maintaining a rather constant ratio. The substance appears in a high concentration in bile, where it facilitates the emulsification and absorption of fats and other lipids.

It has recently been established that the cholic acid of bile is formed from cholesterol by the liver. There is also evidence to suggest that at least some of the closely allied substances present in the body, such as the steroid hormones and the D. vitamins or their provitamins are synthesised in the body from cholesterol.

Cholesterol itself is capable of being synthesised in the body, probably from the simple starting compound of acetic acid. The
body also destroys the substance and eliminates it in the faeces in the form of 7-dihydrocholesterol, coprostanone, coprostenol, colestene and dihydrocoprosterol. Cholesterol is not capable of absorption unless esterified and this is attained by the action of the pancreatic enzyme cholesterase in the presence of bile salts.

The absorption of sterols is a highly specific chemical process. A minute difference in configuration of the compound may determine whether a given compound is absorbed or eliminated, thus, allocholesterol, a mixture of isomers of cholesterol, and dihydrocholesterol, a saturated form of cholesterol, are entirely excreted in the faeces. Osteosterol, a sterol of animal origin, closely related to the vegetable sterol sitosterol, is slightly but unmistakably absorbed while sitosterol is rejected.

Under normal conditions cholesterol and its derivatives are eliminated almost entirely in the faeces, little or none being excreted in the urine.

As previously mentioned the absorption of ingested cholesterol takes place in the bowel under the influence of cholesterase and bile salts. Synthesis of the substance takes place in various situations, the chief of which seem to be the bowel and the liver. There is a definite equilibrium maintained between the synthesis and absorption of the substance so that the total amount of the body cholesterol remains remarkably constant. There is also marked constancy in the individual levels of the serum cholesterol in serial observations in the same person, hence the wide range of values usually quoted as the normal range. The amount of cholesterol ingested by a given individual has little effect on the level of the serum cholesterol. It would appear that the excess of the substance is not absorbed and the original level is thus maintained, or else is transported to the liver and stored in that site.

A large amount of cholesterol is excreted in the bile in the free form and passes into the intestine where it is metabolised to one of the forms excreted or converted to ester and re-absorbed and transported to the liver.

NORMAL CHOLESTEROL RANGE

The normal range of serum cholesterol is usually quoted to be from about 130 to about 240 or 260 mgms%. Some workers state that the upper limit of normal should be as high as 320 mgms% but a total cholesterol level of this magnitude is usually regarded to be in the abnormal range.
The range of the free cholesterol is from about 40-80 mgms%, that is around 30% of the total cholesterol in normal individuals.

The ester cholesterol, which is usually expressed as a percentage of the total cholesterol, falls within the remarkably close limits of 70.3 to 76.9% of the total.

The ranges stated above are those for the Schoenheimer-Sperry method. Other methods have normal values which differ somewhat from those quoted.

**ABNORMAL SERUM CHOLESTEROL**

The serum cholesterol is said to be abnormal in a great variety of conditions, and this has been well established over a long period. The most notable changes are possibly the raised values found in such conditions as diabetes mellitus, obstructive jaundice, myxoedema, xanthomatosis, and in some cases of Type II nephritis. Low values are encountered most commonly in severe anaemia and in a small number of cases of acute thyrotoxicosis.

In infective hepatitis the total serum cholesterol is commonly within normal limits but the majority of the cholesterol is in the free form. This fact thus makes the estimation of the free and ester cholesterol of importance in this condition.

In obstructive jaundice the total serum cholesterol is raised but the ratio of free to ester cholesterol is usually maintained, except in cases where there is concurrent liver damage due to cirrhosis or other hepato-cellular damage.

**CAUSES OF THE CHANGES IN CHOLESTEROL VALUES**

1. **DIABETES**

   The hypercholesterolaemia of diabetes occurs as a result of the abnormal localisation of the lipids in the blood rather than as an abnormality of the lipid metabolism. It may be that the lipaemia of diabetes is a reflection of the increased metabolism of fat because of the unavailability of carbohydrate. The fact that cholesterol can be synthesised from simple carbon-chain compounds such as acetate, raises the possibility of increased production of cholesterol in diabetes as a result of the increased formation of these substances incident to the increased metabolism of fat. It has been shown that hypercholesterolaemia may be produced in diabetic patients by a high fat intake, undernutrition, severe acidosis, and coma.
2. **NEPHROTIC SYNDROME**

The cause of the extreme hypercholesterolaemia in the nephrotic syndrome is quite unknown. The ability to utilise fats is unimpaired and the defect appears to lie in the processes which control the mobilisation of lipids. The level of the serum cholesterol seems to bear some relationship to the amount of oedema, but when the oedema is reduced the cholesterol level often remains high.

3. **OBSTRUCTIVE JAUNDICE**

The high values for serum cholesterol commonly encountered in obstructive jaundice appear to be the result of some interference with the bile acids and other lipid constituents of the bile. The exact cause of the phenomenon is not known.

4. **MYXOEDEMA**

Hypercholesterolaemia is rather constantly associated with the hypothyroid state, the level of the serum cholesterol often paralleling the reduction of the basal metabolism. The cause of this state appears to lie in the fact that there is hyperactivity of the anterior hypophysis secondary to the diminished thyroid function. A return to normal values usually results after the administration of thyroid extract. There is evidence to suggest that the thyroid hormone influences the distribution of cholesterol between the plasma and the tissues rather than the synthesis and the destruction of cholesterol.

5. **XANTHOMATOSIS**

Xanthomas, especially if present in large numbers, are almost always associated with hypercholesterolaemia, and extremely high values may be encountered. The condition is nearly always found in association with diseases in which the serum cholesterol is raised, such as a complication of severe diabetes of long standing, obstructive jaundice or lipid nephrosis. Therefore the causes of the raised values in most cases are those of the primary condition. Primary xanthomas do occur, but are rare and are also associated with hypercholesterolaemia, which appears to be due to some fundamental disturbance of the reticuloendothelial system.

**HYPOCHOLESTEROLAEMIA**

The reduction of the level of the serum cholesterol occurs also in a considerable number of well defined clinical conditions, chief among which are severe anaemia, hepatic disease, infection, hyperthyroidism, inanition and the terminal states.
1. **ANAEMIA**

Hypocholesterolaemia occurs in patients who are suffering from severe chronic anaemias, such as pernicious anaemia, hypochromic anaemia, and the anaemia of leukemia. It does not occur in aplastic anaemia or in severe anaemia following acute haemorrhage. The primary cause of this diminished serum cholesterol appears to be that of simple malnutrition rather than any underlying metabolic cause.

2. **INFECTIOUS DISEASES**

As a general rule the serum cholesterol decreases in the acute febrile stages of infectious diseases. In acute respiratory infections the ratio of the free to ester cholesterol rises, because the deficiency involves the ester fraction almost entirely. The cause of these diminished values in infection is not clearly understood, but is thought to have some relation to the phenomenon of immunity. It is held by some workers that the degree of hypocholesterolaemia is of significance in prognosis of tuberculosis, in which condition it appears to be related to the severity of the process.

3. **HYPERTHYROIDISM**

It is said that there is a rough relationship between the serum cholesterol and the basal metabolism in patients with hyperthyroidism, and that values as low as 60 mgms% may be found in those near the thyroid crisis. The average values in exophthalmic goitre tend to be somewhat lower than those in toxic nodular goitre. The changes appear to be due to an alteration in the distribution of the cholesterol between the tissues and the serum, which in turn is thought to be caused by some activity of the pituitary gland and by some substance excreted by the thyroid itself.

4. **INANITION**

A diminution of the serum cholesterol is commonly noted in conditions associated with wasting and cachexia, but the significance is not clearly understood. In many cases there is an associated hypoproteinaemia, and both states appear to be directly related to the stage of malnutrition.

5. **TERMINAL STAGES**

It has been observed that the serum cholesterol in the terminal stages of a great many conditions falls to very low levels. There is evidence to suggest that this is a result of increased activity of the
cells of the reticulo-endothelial system. During periods of stress, the adrenal cortex is stimulated to increased activity, which is accompanied by a decrease in the adrenal cholesterol. It is possible that under such conditions that the cholesterol stores of the body may be depleted, leading to hypocholesterolaemia. A similar mechanism may operate in acute infection. The fact remains that the development of hypocholesterolaemia under these circumstances is of serious prognostic import, especially in conditions where the cholesterol may have been at a high level previously.

TECHNICAL

METHODS

For the benefit of those who do not have access to the various analytical methods used in the preparation of the data in this paper we propose to give the methods in full, together with some notes on any modifications. The statistical methods employed are the standard methods in general usage.

I. SCHOENHEIMER AND SPERRY METHOD FOR TOTAL ESTER AND FREE CHOLESTEROL.

Blood is obtained by venipuncture without venous stasis, allowed to clot, and the serum separated by centrifugation. If the analysis cannot be conducted immediately, it is essential to inactivate the serum by heat to destroy the pancreatic cholesterase which would otherwise cause alteration in the ratio of the free to total cholesterol. After inactivation the serum may be stored in the refrigerator indefinitely providing infection is excluded. We have found that it is convenient to conduct about six tests simultaneously.

REAGENTS

1. ACETONE-ALCOHOL MIXTURE. Mix 1 part of absolute ethanol with 1 part of redistilled acetone.
2. DIGITONIN SOLUTION. Dissolve 400 mgms of reagent grade digitonin in 100 ml. of distilled water. Filter before use if not clear.
3. 10% ACETIC ACID. Dilute 1 volume of glacial acetic acid to 10 volumes with distilled water and mix.
4. ACETONE-ETHER MIXTURE. To 1 volume of redistilled acetone add 2 volumes of peroxide free ether. To prepare peroxide free ether, wash ordinary ether with sodium sulphite, followed by water, and distil from calcium chloride.
5. POTASSIUM HYDROXIDE SOLUTION. Prepare a 10\% solution of reagent grade potassium hydroxide in distilled water and place in a bottle equipped with a dropper.

6. PHENOLPHTHALEIN SOLUTION. A 1\% alcoholic solution.

7. ACETIC ANHYDRIDE-SULPHURIC ACID REAGENT. Just before needed place 20 ml. of acetic anhydride (A.R.) in a stoppered measuring cylinder and chill in ice water. Add 1 ml. concentrated sulphuric acid in small portions, keeping the mixture cool. Keep the prepared mixture in the ice bath. Do not use any of the mixture more than one hour old.

8. STOCK CHOLESTEROL STANDARD. Dissolve 100 mgms. of pure dry cholesterol in about 50 mls. of glacial acetic acid by warming on a hot plate and stirring. Transfer with rinsings to a 100 ml. volumetric flask, dilute to 100 ml. with acetic acid and mix. This solution contains 1 mgm. of cholesterol in 1 ml. and is stable in the cold.

9. DILUTE STANDARD CHOLESTEROL. Transfer 5 ml. of the stock standard to a 50 ml. volumetric flask and dilute to the mark with glacial acetic acid and mix well. This standard contains 0.2 mgm. cholesterol in 2.0 ml.

PROCEDURE

Place about 10 ml. of the acetone-alcohol mixture in a 25 ml. volumetric flask and add 1.0 ml. of inactivated serum or plasma down the side of the flask below the graduation mark. Swirl the flask to produce a finely divided precipitate. Immerse the flask in a boiling water bath, with swirling to prevent bumping, until the solvent boils. Allow to cool to room temperature and make up to the mark with the solvent. Mix well and filter through a dry filter paper into a dry tube.

PRECIPITATION OF FREE CHOLESTEROL

Transfer 6 ml. of filtrate to a 15 ml. graduated centrifuge tube, add 3 ml. of digitonin solution, and one drop of 10\% acetic acid. Place a stirring rod in the tube and stir thoroughly. Place the tube, together with the rod, in a quart size preserving jar, cover tightly, and allow to stand overnight at room temperature. In the morning, place the tube and rod in a rack, and stir the contents gently to release any particles which may be attached to the walls of the tube. Remove the rod without touching the sides
of the tube and place carefully in a numbered wire rack in such a way that any adherant particles are not rubbed off. Centrifuge the tube for 15 minutes at about 3000 r.p.m. Decant the supernatant and drain in the inverted position for a few minutes. A few particles may float on the top of the supernatant after centrifugation; they can be discarded with the supernatant.

SAPONIFICATION AND PRECIPITATION OF TOTAL CHOLESTEROL

Add 3 drops of potassium hydroxide solution to a 15 ml. graduated centrifuge tube, and add 3 ml. of filtrate. Insert a stirring rod and stir vigorously until no droplets of alkali can be seen in the solution. Place the rod and tube in a preserving jar in which a layer of sand about 3 cm. deep has been previously heated to a temperature of about 45 degrees C. The sand acts as a heat reservoir. Close the jar tightly and place in an incubator at 37 degrees C. for 30 minutes. Remove the tube to a rack, allow to cool to room temperature, remove the rod, and add acetone-alcohol mixture to the 6 ml. mark. Add 1 drop of phenolphthalein followed by 10% acetic acid, drop by drop until the red colour has disappeared. Add 1 drop in excess, followed by 3 ml. digitonin solution. Stir thoroughly, place in a preserving jar, close tightly and allow to stand overnight. Separate the precipitate as for free cholesterol and then proceed for both free and total cholesterol as follows.

WASHING THE PRECIPITATES

After decanting the supernatant fluid, wash down the walls of the tube and the rod with about 1.5 to 2.0 ml. of the acetone-ether mixture, added from a dropping pipette with attached rubber bulb. Stir the precipitate thoroughly and replace the rod on the rack. Centrifuge for 5 minutes. Repeat the washing twice more with ether instead of the mixture. The precipitates are now ready for colour development.

DEVELOPMENT AND READING OF COLOUR

Heat a sand bath to 110-115 degrees C. in an oven or over a hot plate. Place the tubes containing the washed cholesterol digitonide in the pan and maintain at the same temperature for 30 minutes. Remove the source of heat and add to each tube while still in the pan 2 ml. of glacial acetic acid, washing down the sides of the tube in the process. Stir well with the rod and leave in the sand for not longer than 2 minutes to effect solution. Undissolved material at this stage is neglected.

Place the tubes in a water bath at 25 degrees C. and allow to
come to temperature equilibrium. Note the time and add 4 ml. of the acetic anhydride-sulphuric acid reagent, mix well and return to the bath. Allow to stand 27 minutes and read in the colorimeter.

A standard is prepared by placing 2.0 ml. of the dilute standard in a 15 ml. tube and placing in the bath at the same time as the test series. The colour in the standard is developed in exactly the same manner as in the test solutions. For photometric measurement a blank is run, which consists of 2.0 ml. of glacial acetic acid treated in the same fashion as the test and standard solutions.

The colour so produced is read in the photoelectric colorimeter, using a filter with maximum absorption at 625 mu., i.e., a red filter. The instrument is set at the null point with the reagent blank.

**CALCULATION**

Since the amounts of filtrate taken for the free and total cholesterol determinations represent 0.24 and 0.12 ml. of the original sample respectively, the calculations are therefore as follows:

*Free cholesterol:*

\[
\frac{\text{Density of Unknown}}{\text{Density of Standard}} \times 0.2 \times \frac{\text{mgm. of free cholesterol per 100 ml.}}{0.24} = \text{mgm. of free cholesterol per 100 ml.}
\]

*Total cholesterol:*

\[
\frac{\text{Density of Unknown}}{\text{Density of Standard}} \times 0.2 \times \frac{\text{mgm. total cholesterol per 100 ml.}}{0.12} = \text{mgm. total cholesterol per 100 ml.}
\]

II. **KING MODIFICATION OF THE SACKETT METHOD (DIRECT)**

This method has the virtue of being relatively simple, rapid and reproducible, but has the disadvantage of estimating the total cholesterol only. As it also employs the Leibermann-Burchard reaction it too suffers from the same disadvantages as other methods employing this reaction. It is useful, however, to the small laboratory as it requires the minimum of equipment and is not too time consuming.

**REAGENTS**

1. **STANDARD CHOLESTEROL SOLUTION**: Dissolve 0.1 gm. of pure dry cholesterol in 100 ml. of anhydrous chloroform.
2. Acetic anhydride A.R.
3. Concentrated sulphuric acid A.R.
4. Absolute alcohol.
5. Anhydrous ether, A.R.
PROCEDURE

Pipette 0.2 ml. of blood, plasma or serum into a 15 ml. centrifuge tube containing 8.0 ml. of absolute alcohol and 2.0 ml. of ether.

Stopper the tube and shake vigorously.

Allow it to lie horizontally (with the precipitate evenly distributed along its length) for about 30 minutes.

Centrifuge and pour off the supernatant as completely as possible into a dry 50 ml. beaker.

Evaporate the solvent carefully on a hot plate or water bath avoiding overheating.

Wash the residue into a 10 ml. stoppered cylinder with small portions (not exceeding 2.0 ml.) of chloroform. Repeat the washing until all the cholesterol is transferred to the cylinder. Make up to the 5 ml. mark with chloroform.

Prepare a standard by diluting 0.5 ml. of the standard to 5 ml. in another cylinder.

Add 2.0 ml. of acetic anhydride and 0.2 ml. of sulphuric acid to each cylinder. Stopper and mix well, and allow to stand in the dark for 10 minutes.

At the end of the time stated, read the green colours which have developed in the photoelectric colorimeter using a red filter with a maximum absorption at 625 μu.

CALCULATION

\[ \text{Reading of Unknown} \times 25 = \text{gms. total cholesterol per 100 ml.} \]

Reading of Standard

III. METHOD FOR FREE AND TOTAL CHOLESTEROL; ZAK et al. 1954

This method has proved to be rapid, simple and reliable in our small experience with it. It uses the same end point mechanism as the direct method of Zlatkis, i.e. the ferric chloride chromogen which is, as mentioned earlier, more sensitive and stable than the Leibermann-Burchard colour. In our opinion any method which does not use the latter end point is an advantage providing it fulfills the other criteria essential to a satisfactory method, such as accuracy, reproducibility, ease of manipulation and speed. We therefore intend to do further investigation into the use of this method. As it appears to be such a promising method we will give the details below.

REAGENTS

1. Digitonin Solution: Dissolve 1 gm. of digitonin in 50 ml. of ethanol. Dilute to 100 ml. with distilled water.
2. Iron Stock Solution: Dissolve 2.5 gm. FeCl₃·6H₂O in 25 ml. of glacial acetic acid. Store in the freezing compartment of the refrigerator. Thaw when needed. No precipitate forms in the thawed solution.

3. Colour Reagent: Pipette 1.0 ml. of the stock iron solution into a volumetric flask and dilute to the mark with sulphuric acid A.R. Discard when any precipitate becomes evident.

4. Cholesterol Standard: Dissolve 100 mgm. of pure dry ash free cholesterol in 100 ml. of glacial acetic acid.

PROCEDURE
Preparation of working curve: Pipette 0.0, 0.1, 0.2 and 0.3 ml. of the standard into 30 ml. tubes and dilute each to 3.0 ml. with glacial acetic acid. Pipette 2.0 ml. of the colour reagent into each tube, mix and allow to cool. Measure the density of each at 560 mu., and construct a calibration curve from the readings.

ANALYSIS OF THE SAMPLE
Prepare a filtrate of the serum as set down in the method of Schoenheimer and Sperry. Pipette 2.5 ml. aliquots into a 30 ml. test tube and a 15 ml. centrifuge tube. Place both tubes in a boiling water bath and evaporate the contents of the test tube to dryness and the contents of the centrifuge tube to 0.5-1.0 ml.

TOTAL CHOLESTEROL
Pipette 3.0 ml. of glacial acetic acid into the test tube which contains the dry residue and warm about 30 seconds in the water bath to dissolve the residue and remove it from the sides of the tube. Add 2.0 ml. of the colour reagent, mix thoroughly, allow to cool and measure the density of the colour at 560 mu. using a 1 cm. cuvette.

FREE CHOLESTEROL
Add 1.0 ml. of 1% digitonin solution to the partially evaporated contents of the centrifuge tube, wait 10 minutes and centrifuge for 10 minutes at 3500 r.p.m. Decant the supernatant fluid and drain the inverted tube on filter paper for several minutes. Blow 4.0 ml. of acetone into the tube to disperse the precipitate. Mix thoroughly, centrifuge, decant and drain as before. Add 3.0 ml. of glacial acetic acid and warm to dissolve the precipitate if necessary. Add 2.0 ml. of the colour reagent, cool and measure the density of the resultant colour at 560 mu. as before.

CALCULATION
Read the densities obtained from the unknowns against the values on a calibration curve prepared from the readings of the working standards as previously described.
CHOLESTEROL ESTER

To obtain the value for the ester portion of the total cholesterol subtract the value of the free cholesterol from that of the total cholesterol and express the esters as a percentage of the total cholesterol.

OTHER METHODS

Recently two other methods have been described and involve different principles from those already described and although we have no experience with either, the principles are included in this paper for the sake of completeness. One, that of Feichmeir and Bergerman, employs the substance anthrone which does not directly combine with the cholesterol molecule but does so with the digitonin molecule and therefore could be classed as an indirect method. In brief the cholesterol is precipitated as the digitonide and the amount of digitonin in the complex estimated by means of the anthrone reagent. The method claims to be accurate, reproducible, and the end point stable and sensitive. Apart from the anthrone reagent the reagents used are commonly found in the laboratory. This method may well be worth a trial.

The second method is a modification of the Schoenheimer-Sperry method, but instead of the colorimetric end point the method uses a nephelometer and is a turbidimetric one, which leaves it open to the major objection to these methods; the difficulty of reproducing the particle size and the degree of aggregation of the particles. In common with the parent method it is time consuming and it is doubtful if the accuracy is greater.

RESULTS OF THE ESTIMATION OF THE FREE, TOTAL, AND ESTER CHOLESTEROL IN 274 SPECIMENS OF SERUM

Our series of sera on which the analyses were made were obtained in the main from patients of the general practitioners of the Thames area and from in-patients in this hospital. Included in the series are sera from 79 normals which were submitted for cholesterol estimation as a routine investigation. Of the total sera examined there were 195 abnormal in one respect or another, either in the total serum cholesterol or in the proportion of one of the components. Of these a fair percentage had normal total values and the abnormality occurred in the proportion of the free to total cholesterol, which we suspect may be due in part to the failure of inactivating the sera which could not be examined on the day of collection, thus allowing the cholesterase present in the sera to cause some of the free cholesterol to be esterified and give high values for ester cholesterol.
Table I summarises the clinical states of the patients on whom the examination was requested and the findings in each category.

<table>
<thead>
<tr>
<th>Clinical Condition</th>
<th>No. of Cases</th>
<th>Cholesterol Level</th>
<th>% No. of Cases</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Free</td>
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<td>Myxoedema including</td>
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<td>N 22</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>H 3</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L 10</td>
<td>9</td>
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<tr>
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<td>8</td>
<td>N 4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H 3</td>
<td>3</td>
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<td>L 1</td>
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<tr>
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<td></td>
<td></td>
<td>L 12</td>
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<td>Normal</td>
<td>79</td>
<td>N 79</td>
<td>79</td>
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<td>274</td>
<td>N 171</td>
<td>189</td>
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<td></td>
<td></td>
<td>H 61</td>
<td>46</td>
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<td></td>
<td></td>
<td>L 42</td>
<td>38</td>
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*H equals High, N equals Normal, L equals Low.

It will be seen from Table I that there is the possibility of encountering any combination of values in any of the disease states mentioned, although there is a tendency towards certain levels in some well defined conditions such as myxoedema where there is usually a high total value with a normal distribution of the free and ester fractions. Even in this condition low values are sometimes found where the clinical condition and B.M.R. of the patient are consistent with a diagnosis of myxoedema. In well defined hyperthyroidism the serum cholesterol is commonly at or below the lower limit of normal, again with a normal distribution of the free and ester cholesterol, and again high values are also encountered.

Perhaps the most characteristic findings are those associated with parenchymatous diseases of the liver, such as infective hepatitis and cirrhosis. In both these conditions it is common to find that the total cholesterol value is at or below the lower limits of the normal range, and equally common to find that the free cholesterol
level is very high, even as much as 80 to 90% of the total, and further that the ester cholesterol is subsequently reduced to comparably low levels, sometimes to such an extent as to be almost entirely absent. These changes are most marked when the condition is in the acute stage or when there is an exacerbation of the disease.

The changes in the serum cholesterol fractions occur at about the same time as the changes are noted in the empirical liver function tests and provide useful confirmation of the extent of the liver damage as estimated by the other tests commonly performed. In our opinion the estimation of the total, and differentiation of the cholesterol fractions is a useful adjunct to the usual liver function tests normally available in the clinical laboratory. The test undoubtedly is most useful as a means of assistance in the differentiation of obstructive jaundice from infectious hepatitis which is not always clear from the estimation of the phosphatase and serum bilirubin levels.

Marked changes in the cholesterol fractions were also observed in the four cases of diabetes mellitus in which the estimation was done. In all cases the value of the total cholesterol was raised, in one case to 412 mgm%, and the ratio of the fractions was within normal limits, thus confirming the findings of other workers.

**RANGE OF VALUES**

Of the total number of sera examined, 171 were found to have values for total cholesterol within the normal limits, but of these only 79 had normal values for both free and ester cholesterol. Total cholesterol was above the normal figure of 235 mgm% in 59 sera, and below the normal in 44 sera. The highest value was obtained in a case of diabetes mellitus (412 mgm%) and the lowest in a case of aleukemic leukemia which had run a chronic course for nine months, the value in this case was 69 mgm%.

**COMPARISON WITH A DIRECT METHOD**

The total cholesterol was estimated simultaneously on 224 of the specimens of serum by means of King's modification of Sackett's method. The results of these duplicate estimations of the same sera by two different methods show that the "direct" method tends to give a higher value for the total cholesterol when compared with the Schoenheimer-Sperry method. The difference was not entirely on the positive side, however, and a "scattergram" prepared from the results of both methods shows that the correlation between the two methods was not good and tended to err on the positive side of the theoretical 45 degree line of complete agreement.

Statistical analysis of the results of the estimations by the Sackett method which were within the normal range showed a sta-
tistical mean of $204.4 \pm 84.6$ (SD) mgms% with a probable error of the mean of 4.41 mgm%, and over the whole range of the values found a mean of $268.5 \pm 152.3$ mgms%.

Similar analysis of the Schoenheimer-Sperry results of 79 sera from normal individuals showed the following values:

<table>
<thead>
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<th></th>
<th>Total</th>
<th>Free</th>
<th>Ester</th>
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</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>193.5 mgm</td>
<td>50.06 mgm</td>
<td>73.92%</td>
</tr>
<tr>
<td><strong>PE Mean</strong></td>
<td>1.72 mgm</td>
<td>8.58 mgm</td>
<td>2.00%</td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td>22.68 mgm</td>
<td>0.66 mgm</td>
<td>0.77%</td>
</tr>
</tbody>
</table>

From the series of estimations of the sera from normal patients the normal ranges of the total, free and ester cholesterol should fall between the limits of the mean ± 2 SD in 19 out of 20 cases, i.e. total cholesterol 146.1-231 mgm%; free cholesterol 32.9-67.2 mgm%; and ester cholesterol 69.9-77.9%. These figures agree closely with the values quoted by the originators of the method and were largely substantiated in the present series.

By similar methods the values of the Sackett estimations would be expected to fall within the same range of the mean ± 2 SD which would give a normal range of 35-374 mgm% in this series, a result which is clearly ridiculous.

In our hands the Sackett method proved to be unsatisfactory as compared with a method of proved reliability such as the digitonide methods. The greatest advantage of this method is that it is rapid and requires a minimum of chemical skill and uses common reagents. The great disadvantage is that the method of extraction appears somewhat haphazard and liable to introduce errors. The end point is very sensitive and a large amount of colour is developed rapidly, but as is common with all methods employing the Leibermann-Burchard reaction, is unstable.

The Schoenheimer-Sperry method, on the other hand, has proved reliable in our hands. The method suffers from the disadvantage that it is somewhat time consuming, and requires more than usual attention to technical details to ensure reliable results. The use of digitonin may appear a difficulty to some as this substance is very expensive and is not easy to obtain from laboratory supply houses. A considerable number of manipulations are required in the course of the estimation and unless great care is exercised these could introduce gross errors. The final colour of the solution is not very dense and a sensitive instrument is necessary to obtain consistent readings and the production of colour must be very closely controlled.

**CONCLUSIONS**

From our observations it appears to us that where the estimation of the serum cholesterol is undertaken it is desirable to also
estimate the cholesterol fractions. The choice of method to this end is not great but from our experience the Schoenheimer-Sperry method is as satisfactory as any with the possible exception of the newer ferric chloride method of Zak with which we have had limited experience.

In regard to the logic of the necessity of estimating the ratio of the free and ester cholesterol, we have found that on innumerable occasions while the total cholesterol level was quite normal the ratio of the free and ester fractions was grossly disturbed and that their estimation frequently enabled the physician to gain a more precise assessment of the patient's condition. This especially applied in cases of jaundice.

It would seem that the estimation of the serum cholesterol is of some value to the clinician in a number of conditions and that it may well give a good clue to the correct diagnosis in some cases. It must be emphasised that the estimation of this substance can by no means be the criterion of the diagnosis of any given condition, a feature which it shares with most of the tests performed in the clinical laboratory.

It must be further emphasised that the wide range of the normal values makes for some difficulty in the interpretation of the findings in a given case and small fluctuations from these are of little importance. As is well known the cholesterol level in normal subjects varies over a wide range, but the level for a given individual remains remarkably constant in health. It follows, therefore, that where the normal level for a patient is about 150 mgm%, a level of 230 mgm% could well be abnormal although this is still within the range given as normal for this particular patient. To be of real diagnostic assistance the cholesterol estimations should be done on more than one occasion and any variation observed.

In regard to the methods available for the estimation of the serum cholesterol, we feel that any method which employs the Leibermann-Burchard reaction as a colour producing system is not entirely satisfactory as this reaction is unstable and must be closely controlled to achieve comparable results. The reaction is sensitive to temperature, light, and the proportion of acetic anhydride to sulphuric acid used in the reaction. If a satisfactory alternative end-point method can be perfected a major advance in the estimation of cholesterol will have been attained. The methods using acidic ferric chloride and the anthrone reagent respectively appear to offer suitable alternatives in this direction. We hope to investigate the possibilities of both these methods in due course in another parallel series with an established method.
SUMMARY

A résumé of the metabolism of cholesterol and the variations of the level of this substance in various conditions has been presented.

Three methods for the estimation of the serum cholesterol have been described in detail.

The results of a considerable number of tests conducted in parallel by two different methods and a comparison of the results obtained is made.

Our observations on the relative merits of these methods have been presented.

A survey of the results in various disease states has been made and the conclusions reached outlined.

ACKNOWLEDGEMENTS

We wish to express our thanks to Dr. K. R. Archer, Medical Superintendent of this hospital, for his permission to publish this paper, and for his helpful criticism.

Thanks are due also to the General Practitioners of this area for their help in making many of the sera used in this survey available.

REFERENCES

A CASE OF MONOCYTIC LEUKAEMIA
MISS J. A. MAITLAND
(Christchurch Public Hospital)

Monocytic leukaemia is a comparatively rare disease, and not many cases have been recorded. Most are acute, or subacute, and the patients seldom live more than three or four months from the onset of the disease, although during this time they may have several remissions, when their blood counts will appear relatively normal. There have, however, been some instances of chronic monocytic leukaemia with a duration of four to six years.

The clinical features are similar to those found in other leukaemias, with the spleen and liver generally enlarged and the patient showing some degree of anaemia. But a symptom associated more with monocytic leukaemia is hypertrophy of the gums, with numerous sloughing areas, and often bleeding and ulceration. This

*Upper Left:* An immature monocyte at onset of disease.
*Upper Right:* Very immature cells in terminal stage of the leukaemia.
*Lower:* Hypertrophy of the gums characteristic of the disease.
mouth condition may disappear during remissions, but in the terminal stages of the disease becomes extremely distressing.

The blood picture is usually one of moderate leucocytosis, with a predominance (50-90%) of monocytes, mostly mature at the onset. As the disease progresses more immature forms are to be found, many with pseudopodia. During remissions the total leucocyte count may be within normal limits with only 20-30% monocytes. Platelets are generally greatly reduced.

At the end of March, 1954, a woman aged 55 was admitted to hospital, with a history of severe chest pain, breathlessness, sore throat, fever and nausea, all of a few days' duration. Provisional diagnosis was one of right basal pneumonia and pleurisy.

A blood "screen" test was carried out the next day with the following results:

- Haemoglobin: 11.5 gms/100 mls.
- P.C.V.: 34%.
- Leucocyte count: 37,000/cu.mm.
- Polymorphs: 10%-3,700/cu.mm.
- Lymphocytes: 15%-5,550/cu.mm.
- Monocytes: 75%-27,750/cu.mm.

The red cells appeared normal and platelets were scanty in the stained film. The monocytes were mostly mature, but there were occasional young forms and a few blast cells. The diagnosis was one of acute monocytic leukaemia and a bone marrow puncture was indicated.

This was done several days later and the films showed about 80% monocytes and monoblasts. The monocytic series was highly abnormal, ranging from very immature to completely normal cells. Frequently mitosis was observed and there was a high proportion of immature cells. The bone marrow picture confirmed the earlier diagnosis of an acute monocytic leukaemia.

The patient was treated with penicillin for five days and codein was given to relieve the pain which continued to be severe at times, extending right down to the abdomen.

Within a week the total leucocyte count had reverted to normal, with only 34% monocytes, and the patient's general condition improved, although areas of pigmentation (purpura) developed on her legs and her gums became rather painful. At the end of a fortnight she was discharged.

A fortnight later she was readmitted with severe chest pains and abdominal discomfort. The pigmented areas covered her arms as well as her legs. On further examination the liver and spleen were found to be grossly enlarged and her gums severely infected. There were numerous haemorrhagic areas on her gums and soft palate. The leucocyte count had risen again with a
high proportion of monocytes, and the haemoglobin was down to 9.5 gms/100mls. A transfusion of two pints of blood temporarily improved her condition, but within a week her condition began to deteriorate again; the gum hyperplasia became more marked, and the purpuric rash appeared to be spreading. Cortisone treatment had been started during this time and a further course of penicillin was also given. The patient had a brief remission lasting about a week, during which time the purpura disappeared, but another haemoglobin indicated the need for a further transfusion of two pints of blood.

The patient’s gums were still extremely tender and she was advised to have her teeth extracted. This was carried out and the gums healed quite satisfactorily. A strip of hypertrophic tissue from the gums was sent to the Histology laboratory for examination and the tissue was found to be extensively infiltrated and destroyed by large numbers of mononuclear cells. Silver impregnation showed a fine reticulum surrounding the mononuclear cells, and the general appearance was that of reticulosarcoma.

Some few weeks later the patient’s haemoglobin had dropped to 7.5 gms/100mls but after a transfusion of three pints of blood she improved considerably and a week later was discharged from hospital after a stay of almost three months.

During the next three months she was in and out of hospital several times and received transfusions of packed cells each time. On one occasion her haemoglobin was as low as 3.5gms/100 mls but the transfusions helped her greatly and she was only in hospital for a week or so at a time. While at home her health was quite satisfactory, although she was subject to occasional fainting bouts, and often had severe chest pains.

A few days before Christmas the patient was admitted to the ward in considerable distress and a blood count revealed that her leucocytes had soared to 280,000/cu.mm, with 91% monocytes, most of them grossly abnormal. Her haemoglobin was 7gms/100mls and a further transfusion was necessary. Within a week the leucocytes had dropped back to 81,000/cu.mm, with 63% monocytes, but there were now 30% blast cells present, indicating that the leukaemia was in its terminal stage. Electrolyte determination showed a marked potassium deficiency (2.0 m.Eq./1) and the patient was given potassium chloride orally. Cortisone treatment was being continued during this time.

Marked deterioration in the patient’s condition was soon apparent—her gums became extremely painful and she lost all sensation of taste. The severe chest pains were more frequent and her limbs became numb at times. A last blood count showed the leucocytes to be increasing in number with a high percentage of blast cells still present.
This then is an acute monocytic leukaemia, a brief, distressing and inevitably fatal disease. The patient in this case had quite a number of remissions, some of them lasting as long as a month, and during these she was discharged, feeling quite well and strong. On several occasions she was seen riding a bicycle, which indicates how well she must have felt. However, she did spend long periods in hospital and was subject to much pain and discomfort.

The blood counts give us an idea of the progress of the disease over the ten months that the patient was known to be leukaemic. The monocytes, mostly mature at first, gradually became more and more immature and atypical as the months passed.

During remissions the leucocyte count was generally lower than at other times, with the majority of monocytes appearing normal although still many in number. Occasional blast cells were noted in almost all the differential counts but a week before the patient died the blasts had risen from 3% to 30% indicating the final acute stage. The progressive anaemia associated with the disease was checked by repeated transfusions both of whole blood and packed cells.

Cortisone treatment was given in the hope of slowing down the proliferation of the leucocytes and this possibly induced some of the remissions. Penicillin treatment was almost continuous and afforded the patient some degree of protection from any infection she was liable to contract through a reduced polymorph count.

This proven case of acute monocytic leukaemia is the first the Christchurch Hospital has had for a number of years and the clinical features and blood counts, all indicative of the course of the disease, were followed with great interest by members of the staff.

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VIRUSES GENES AND ENZYMES

B. McLEAN

Pathology Department, Napier Hospital

(Winner Essay Section, Junior Essay Competition, 1955)

In modern laboratory practice biochemistry is assuming greater importance than bacteriology, and diagnosis depends more and more on biochemical tests performed in the laboratory. This is due to the advances made in biochemical research, and to the gradual conquest of bacterial infections with a resultant concentration of investigation on diseases due to other causes.

The clinician and laboratory biochemist are concerned with the physiology of humans, but investigation has been carried out on single homogeneous cells, rather than on multicellular organisms, since the former is the study of life at its most universal and fundamental level. No matter to what grade of organisation or specialisation organisms may belong, they are basically composed of the same universal living substance called protoplasm.

Life exists in the chemical and physical relationships between the materials which constitute protoplasm. There is a state of ceaseless chemical activity to the sum total of which is applied the term metabolism. This consists in the synthesis and accumulation of material, the storing up of energy, and the conversion of potential energy into the kinetic energy with which work is done.

Chemically, protoplasm consists of proteins, fats, carbohydrates, inorganic salts, gases and water. Of these the most important are the proteins. Enzymes, the catalysts essential for every metabolic process, are proteins. They act together as coenzyme systems having specific effects on certain cellular constituents.

An example is the adenylic system which is required for the splitting off or taking up of phosphate. This system catalyses the probable reaction between adenosine triphosphate and the proteins, actin and myosin. There is much energy liberated in the splitting off of the phosphate and this is converted to work in the form of muscular contraction.

Two other enzyme systems are cozymase, which is responsible for oxidation and reduction, and carbozylase, which catalyses the splitting off of carbon dioxide.

It has been found that vitamins are all essential parts of enzyme systems, and thus their necessity for growth and reproduction is explained. Vitamins are also nucleoproteins. Many of the enzymes and proteins have been isolated and obtained as crystalline proteins. The knowledge of their chemical structure, and their action, has led to the chemotherapy of the sulpha drugs. It was discovered that if a slight alteration was made in the molecular configuration of a vitamin, then the resultant chemical would still
react with the rest of an enzyme system, but would not give the normal end product, thus effectively blocking the whole system. Many bacteria require the vitamin para-aminobenzoic acid. The sulpha drugs closely simulate this substance in molecular configuration. If they are present in great enough and relatively constant concentration, they will act with enzyme systems in place of the vitamin, or rather, in "competition" with it, and thus block the enzyme activity. The bacteria are then more easily overcome by normal bodily defences.

It has been accepted for many years that the controlling influence behind all the physico-chemical reactions which go to make up cell metabolism lies within the cell nucleus. The nucleus also controls the transmission of hereditary traits. Heredity is controlled by particles of living matter, transmitted by both parents to their offspring, and reassorted in each generation. These particles are called genes and are found in linear arrangement on the chromosomes of cell nuclei. The action of genes on substances responsible for certain traits, such as colour and size, causes the reproduction of these traits. Since millions of highly differentiated cells may arise from a fertilised ovum, then the genes influence the metabolic activities of cells specialised for very different functions. Though the mechanism of this control is unknown some connections between genes and life processes have been demonstrated.

Moulds when treated with X-rays and other mutation-inducing agents lost the ability to synthesise some vitamins and could not grow on ordinary media. It has been suggested that enzymes are manufactured as a by-product in the self-reproduction of genes so that mutant genes will not produce normal enzymes.

Chromosomes were found to contain desoxyribonucleic acid (DNA), a complex nucleoprotein not found outside the cell nucleus. The DNA content of each cell is constant for one species and extracted DNA is specific for each species. For example, there is the experiment done on pneumococci. Type I encapsulated pneumococci were cultured to produce a strain which had lost the capsule. These were then treated with DNA extracted from type III encapsulated pneumococci. The resultant bacteria were converted to type III pneumococci, thus demonstrating that DNA is specific and controls hereditary traits.

The chemical structure and molecular configuration of DNA has recently been discovered. The complex molecule is made up of two long chains of alternate phosphate and pentose sugar groups. To each pentose sugar molecule is attached one of four bases—adenine, guanine, cytosine, or thymine—and each of these is bound to a base on the other phosphate sugar chain.

This part of the structure is common to all DNA but the
sequence of bases appears to determine the specific character of some particular DNA. The sequence of bases must apparently control the sequence of amino-acids which constitute the polypeptide chains of the proteins produced in the cell.

Another nucleoprotein which has a part in metabolism is ribonucleic acid (RNA). This substance is very similar in structure to DNA but is found mainly in the cytoplasm. It occurs in varying amounts in different cells according to their metabolic activity. It is known to be concerned with protein synthesis and is present in greatest concentration in cells engaged in protein manufacture. Glandular cells are an example. RNA has been observed, microscopically, to pass from the nucleus to the cytoplasm, and it is thought that in this way the nucleus controls cell metabolism.

Other substances which have been obtained as crystalline nucleoproteins are viruses. Certain plant viruses are very similar in structure to DNA. Viruses behave as living organisms in their ability to grow and reproduce themselves, and to use enzyme systems. Since DNA acts similarly and constitutes a gene some workers have called viruses "naked genes," supposing that a virus is a gene which has undergone spontaneous mutation, and escaped from the control of the chromosome.

When a gene undergoes mutation it will react in cell metabolism in the same way as a virus, producing, of course, abnormalities in the cell. From this idea has re-arisen the theory that cancer is caused by virus. Cancer cells are mutant cells and since, following the above theory, a virus is a mutant gene, then a cancer causing virus may be said to have arisen in the cells. This idea gives a new conception to the term virus. Since the DNA in cancer cells is specific for these cells, then if chemicals can be found which are selectively injurious to this DNA, a chemotherapy for cancer seems possible. Results so far achieved in this new field have been described in a recent N.Z.A.B. journal. An intermediate substance in the manufacture of DNA has been prepared and found to be antagonistic to mutant cells but not to the normal cells. When all the steps in the growth and reproduction of DNA are known then this chemotherapy will no doubt become a reality.

In studies on spontaneous and induced cancers in mice some interesting and similar results were produced. By careful inbreeding, strains were obtained which resisted the action of carcinogens (cancer or mutation inducing agents). Something in their genetic structure must prevent mutation of other genes. Since the specificity of genes depends on the DNA then when the knowledge of the process of DNA's construction is more complete some DNA may
be found which will help in the production of other antineoplastic chemicals. Perhaps preventative chemicals may even be discovered.

The chemical structure and molecular configuration of enzymes, DNA, and other cellular proteins, and their chemical activity, is the basis of metabolism. As has been shown biochemical research has produced many startling results and it is to be expected that most of the problems in the explanation of life processes will eventually be solved in this field of investigation. A complete knowledge of the steps and intermediary substances in the reproduction of genes, in the synthesis and action of enzymes and proteins, and in the conversion of released energy into work, is the aim of investigators. It will produce new concepts, and new means of prevention and cure of diseases. Whether present day theories will be sustained or supplanted remains to be seen.

Though such work seems to be a far cry from the measurement of phosphatase activity in a hospital laboratory, reports of the latest progress make interesting and useful reading for the technician. By-products of investigation will also lead to the production of new techniques and tests, such as the testing of sensitivity of neoplastic tissue cultures to chemical substances mentioned in the N.Z.A.B. Journal.

A full understanding of the work being done is beyond the reach of the laboratory worker but he should be aware of its importance and be acquainted with the fundamental principles involved.
ABSTRACTS

STUDIES ON THE POLIOMYELITIS EPIDEMIC OF 1952-53
A. M. Murphy

Laboratory methods used in the isolation of two strains of poliomyelitis virus serological type II are described. Neutralisation tests were also carried out on six convalescent cases and type II neutralising antibodies were shown to be present in each case.

STUDIES ON THE EPIDEMIOLOGY OF POLIOMYELITIS IN NEW ZEALAND
A. M. Murphy

A survey for the presence of neutralising antibodies to poliomyelitis viruses in the sera of 100 children was carried out. Details on tissue culture and neutralisation tests are given. 79 of the 100 sera examined had antibody to at least one of the three virus types. It is suggested that the 1947-48 epidemic was caused by a type I virus and the 1952-53 epidemic by type II. The results of the survey are discussed in relation to the epidemiology of poliomyelitis in N.Z. and to future vaccination programmes.

POLIOMYELITIS IN NEW ZEALAND, 1952-1953
F. S. Maclean

This article discusses the incidence and distribution of 1298 cases of poliomyelitis occurring in N.Z. during the two years 1952-53. Severity and extent of paralysis is recorded and control measures are discussed. Various epidemics are compared.

PRACTICAL CONSIDERATIONS OF DISEASES OF PORPHYRIN METABOLISM
PORPHYRIA AND PORPHYRINURIA
F. Wm. Sunderman, Jr., M.D., &
F. Wm. Sunderman, M.D.

Current concepts of the metabolic pathways of the porphyrins have been reviewed. A procedure for the detection and identification of the porphyrins in urine and faeces adapted for use in clinical laboratories has been described. The classification of the porphyrins advanced by various authors are discussed. A practical classification, based on the authors’ experience is proposed and illustrative cases are reported. The pathologic and physiologic conditions that may be associated with porphyrinuria have been summarised. A comprehensive list of references to related works is supplied.

THE USE AND LIMITATIONS OF FILTER-PAPER ELECTROPHORESIS
N. H. Martin and G. T. Frangler

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Printed by Simpson & Williams Ltd., 169 St. Asaph Street, Christchurch.