this, such as using an end of the jejunum or a tube made from the greater curve of the stomach. None of these methods has yet reached the orthodoxy of a set piece.

Conclusion

Finally, let me take stock of our position. We have got to the stage of attaching symptoms to an organ, and we believe that it is inflammation of the organ that causes them. This union is due to the surgeons, but before the physician can make proper use of it he must know how often these symptoms do in fact mean what we think. We need a wide endoscopic survey of cases of chest pain and heartburn in the way that Chevalier Jackson foreshadowed. We have also got to the stage of discussing the pathological mechanisms that cause this change. Our views are mostly based on the investigation and treatment of advanced cases, and we may hope that further experience will give us clearer knowledge of what goes wrong in the early ones.

DETERMINATION OF CHOLINESTERASE ACTIVITY IN HUMAN BLOOD

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In a memorandum on the signs, symptoms, and treatment of cases of poisoning resulting from the absorption of the organo-phosphorus insecticides, the British Medical Association in 1951 drew attention to the value of a knowledge of the level of blood cholinesterase in making the diagnosis and following the recovery from the effects of poisoning. Following the work of Callaway, Davies, and Rutland (1951) on the levels of cholinesterase in a normal population, Barnes and Davies (1951), using these results, have shown that lowering of the blood cholinesterase levels could be detected in workers exposed to these insecticides who showed no clinical symptoms.

The estimation of the blood cholinesterase is not a standard procedure in clinical pathology, and it is the purpose of this paper to summarize the available methods for making those determinations and to describe in detail a method suitable for adoption in a general clinical laboratory.

Although "parathion" and "schradan" are poor inhibitors of cholinesterase *in vitro*, they both owe their poisonous properties to the fact that after absorption in the animal body they are converted into active inhibitors of this enzyme (DuBois *et al.*, 1950; Gardiner and Kilby, 1950; Aldridge and Barnes, 1952), and the symptoms of poisoning so produced seem to follow from the inhibition of cholinesterase. There is evidence that the blood cholinesterase is lowered before that in the tissues (Hobbiger, 1951), and it is the loss of the cholinesterase at nerve endings and in the central nervous system that is responsible for the symptoms of poisoning.

Determination of cholinesterase levels in blood can be of value because: (1) The early symptoms of anti-cholinesterase

poisoning are almost wholly local in character and are not necessarily indicative of systemic absorption. Systemic absorption is much more insidious, and the only convenient measure of such a process is the level of cholinesterase in the blood (Davies *et al.*, 1951, unpublished results). (2) A fall in the cell or plasma cholinesterase level may well be the first indication of absorption of these substances (Barnes and Davies, 1951). (3) After poisoning, the blood enzymes will remain at a very low level until the regeneration of new enzyme, and this is very slow. The red-cell enzyme reappears at approximately 1% a day, although the plasma enzyme reappears more quickly (Grob *et al.*, 1947). During this period, when the blood enzymes are low, an individual is hypersensitive to subsequent doses of anti-cholinesterase (Callaway *et al.*, 1951). (4) Many of the symptoms of poisoning by anti-cholinesterase agents are commonplace in character—headache, tightness of chest, nausea, internal discomfort, etc. Hence the determination of cholinesterase blood levels may well be of much value in the differential diagnosis in cases of mild poisoning.

Determination of the Cholinesterases in the Blood

The cholinesterase in human erythrocytes differs from that present in the plasma. These two enzymes possess quite different properties; a knowledge of the properties is desirable in order to understand fully the problem of their assay. This aspect has been well reviewed by Whittaker (1951).

In view of the lack of sensitive or specific methods for the determination of the acetic acid or choline produced during the hydrolysis of acetylcholine by the cholinesterases, most methods have been based on the unspecific measurement of an acid produced.

Warburg Manometric Technique.—This technique, developed by Ammon (1933), is based on the measurement of carbon dioxide liberated from a bicarbonate buffer by the acetic acid liberated by hydrolysis of acetylcholine. Details of this method are available in several recent publications (Adams and Whittaker, 1949; Aldridge, 1950; Callaway *et al.*, 1951). Although it is a most elegant and accurate method it is more suited to research work than the routine determination of large numbers of specimens. The apparatus is expensive and the technique rather specialized.

Continuous Titration Method (Stedman *et al.*, 1932).—In this method the acetic acid formed is continuously neutralized by the addition of alkali. The pH of the reaction system is thus maintained constant. Although the apparatus required is found in most laboratories (pH meter, microburette, constant-temperature bath) and the procedure is simple, only one determination may be carried out at a time, and the procedure is very tedious. It is not recommended for routine estimations.

Colorimetric Method of Hestrin (Wilson and Bergmann, 1950; Diggle and Gage, 1951).—Hestrin (1949) applied a method for the determination of esters to the determination of acetylcholine. Cholinesterase activity may therefore be determined from an estimation of the acetylcholine present before and after enzymic hydrolysis. This method suffers from the inaccuracy usually associated with methods based on the measurement of small differences in relatively large amounts. Also the rate of removal of acetylcholine is not a linear function of enzyme concentration.

Electrometric Method of Michel (1949).—This is essentially an indirect method of measuring cholinesterase activity by the fall in pH of a barbitone/phosphate buffered system due to the acid produced. This empirical buffer system has been devised so that the fall in pH is linear with time even though the activity of the enzyme is being lowered by the fall in pH itself. The method has been shown to correlate well with the standard Warburg technique (Hamblin and Marchand, 1951). The apparatus required is a pH meter reading to 0.01 unit and a constant-temperature bath. The actual procedure involves only two pH readings, one

before and one after hydrolysis for a given time. Since up to 40 estimations may be carried out together, the method is well suited for routine determinations. Hitherto all analyses have been carried out upon cells or plasma (Callaway *et al.*, 1951). This is not absolutely necessary, for, despite differences in the two enzymes, determinations upon whole blood may be satisfactorily used for the routine detection of the possible systemic absorption of the anticholinesterase insecticides. In the event of a given specimen yielding a low result, a venepuncture sample should be obtained and the cells and plasma separately examined.

Experimental Details of the Electrometric Method (Michel, 1949)

Reagents

1. Buffer No. 1 (for erythrocytes):

0.02 M sodium barbitone	4.1236 g.
0.004 M potassium dihydrogen phosphate ..	0.5446 g.
0.60 M potassium chloride	44.730 g.

Dissolve the reagents in 900 ml. of distilled water and determine the pH. Add 0.1 N HCl until pH 8.10 is reached at 25° C. (approximately 15 ml. is required). Dilute to 1 litre.

2. Acetylcholine substrate for erythrocytes (0.11 M). Acetylcholine chloride, available from Roche Products Ltd. in 0.1-g. ampoules, has been found suitable. Dissolve 1 ampoule in 5 ml. of water.

3. Buffer No. 2 (for plasma and whole blood):

0.006 M sodium barbitone	1.2371 g.
0.001 M potassium dihydrogen phosphate ..	0.1361 g.
0.30 M sodium chloride	17.535 g.

Prepare as for buffer No. 1 for erythrocytes, but in this case adjust the pH to 8.0 (approximately 11–12 ml. of 0.1 N HCl is required).

4. Acetylcholine substrate for plasma and whole blood (0.165 M). Dissolve 0.1 g. (1 ampoule) in 3.3 ml. of water.

5. 0.01% w/v saponin.

6. 0.90% w/v sodium chloride.

7. Potassium oxalate as anticoagulant.

Equipment

The minimum equipment required is a pH meter reading to 0.01 unit, a constant-temperature bath at 25° C., and a quantity of wide-bore medium-walled short tubes of 20 ml. capacity. However, for routine examination of many samples it has been found convenient to have an attachment which allows the quick and yet safe movement of the glass electrode from one tube to another. A slight scratch on the glass electrode will cause irreparable damage.

1. *pH Meter*.—Any standard instrument which may be read to 0.01 unit is suitable. We use a Cambridge battery-operated model.

2. *Electrodes*.—Calomel half-electrode Muirhead type with a long flushing tube, and a dip-type glass electrode with a 3-ft. (0.9-m.) lead.

3. *Constant-temperature Bath*.—An ordinary laboratory rectangular water bath with a temperature control of ± 0.2 degree and capable of being operated at 25° C. is quite adequate. The bath is fitted with an electrode-holder which can be moved sideways as well as forwards and backwards. It consists essentially of two pillars, AA (see Diagram), connected by a rod, B. The bottom of each pillar is bent at right angles. A piece of angle brass helps to form with the bottom of the pillar a sliding bracket which permits backward and forward movement of the holder across the width of the bath. A slotted bracket, which carries a vertical rod, C, capable of lateral movements, also carries the actual bracket which supports the holder, D. This can be moved vertically on the rod C. This device permits the controlled movement of the electrode in three dimensions: it is necessary in order to protect the electrodes, which may easily suffer irreparable damage by rough contact with the sides or bottom of the reaction tubes.

Diagram of movable electrode-holder.

4. *Tubes*.—To facilitate the easy and safe manipulation of the electrodes in the reaction tube, tubes 2 in. (5 cm.) high and 1½ in. (3.1 cm.) in diameter, with medium-thick walls and constructed of soda glass, were used. (These can be obtained from Messrs. Hearson and from Messrs. Baird and Tatlock.)

Collection and Preparation of Blood Samples

Provided the blood is not clotted the cholinesterases are quite stable in whole blood. If analyses on erythrocytes and plasma separately are required no haemolysis should be present, and the cells should be separated without delay—preferably within 24 hours.

For whole-blood analyses enough blood may be obtained from finger-tip or ear-lobe punctures. However, for a determination on erythrocytes or plasma a venepuncture is required to obtain the necessary 5 ml. Details of the precautions necessary to obtain samples free from any of the defects mentioned above are given by Harrison (1943).

For the separation of erythrocytes and plasma the following standard procedure is recommended: Centrifuge approximately 5 ml. of oxalated blood at 2,000 r.p.m. for 15 minutes (800 g). Separate plasma and wash cells twice with saline, finally centrifuging for 20 minutes at 800 g. Remove supernatant, thus leaving a preparation of washed cells which have been packed under standardized conditions.

Procedure for Enzyme Estimation

As mentioned above, the method is precisely the same for whole blood, cells, and plasma. The only differences are to be found in the composition of the buffer solution and the concentration of the acetylcholine solution. The following procedure is for either whole blood or plasma; the necessary modifications for cells are included in parentheses: (1) 0.1 ml. of blood, plasma, or cells is measured in an appropriate McClean's blood pipette and transferred to 10 ml. of a mixture of equal volumes of buffer No. 2 (or buffer No. 1 for cells) and saponin solution contained in a reaction tube in the constant-temperature bath at 25° C. (2) Add 1 ml. of 0.165 M acetylcholine chloride (0.11 M for cells). (3) Note the time, and exactly half a minute after the addition of substrate note the pH. (4) Exactly 60 minutes later take the pH reading again. (5) The difference in pH readings, $\Delta \text{pH/hr.}$, is a measure of enzyme activity, and is called the "cholinesterase number" (ChE No.) which is the fall in pH produced by 0.1 ml. of whole blood in one hour $\times 100$.

Normal Limits of Whole-blood-cell and Plasma Activity

The interpretation of figures obtained by any of the procedures outlined above is dependent upon a knowledge of the normal variation of these enzymes in health.

For whole-blood analyses—that is, for determination of the ChE No.—data are available only upon young male

Service personnel between the ages of 18 and 30 (Davies *et al.*, 1951, unpublished results). Figures have been obtained from 230 males from the armed Services.

The mean value of the ChE No. was 111. (The coefficient of variation of this series was 12.1.) Thus the normal limits—that is, the fiducial limits—become 87 and 136. Owing to the difference between the red-cell activity of the Service and civilian groups (Callaway *et al.*, 1951), these values are probably too high by about 5 to 7%, and accordingly a sounder estimate of the lower limit may be taken as 80.

No observations have been made upon the "personal" variation of the ChE No. The relevant data of Callaway *et al.* (1951) may, however, be used to provide an estimate of what must be the difference between two determinations in the same person of the cell (or plasma) enzyme which will indicate departure from normal limits. The differences referred to above for the red-cell and plasma enzyme respectively are 15 and 20% of the higher of the two values. Thus even if the plasma figures were applied to the ChE No.—that is, even if the wider limits were applicable—there still remains a substantial margin between the recognition of abnormal deviation and the onset of those symptoms which indicate the development of lethal processes. The ChE No. must certainly be decreased to below 40% of its initial value before any definite clinical symptoms due to systemic absorption occur (Davies, 1951, unpublished results).

Normal limits for the whole-blood, red-cell, and plasma enzyme are summarized in the Table.

	Cholinesterase Number	
	Lower Limit	Upper Limit
Red blood cells	51	100
Plasma	40	100
Whole blood	80	129

Variations in Blood Cholinesterase Due to Disease

There are many references in the literature to the variation of both cell and serum cholinesterase. Most of the information, however, has not been really substantiated, and in only two conditions is there clear-cut evidence correlating a lowered cholinesterase with the disease. Thus the position has been accurately summed up by Augustinsson (1948) in these words: "The cholinesterase concentration of the blood has been widely studied in order to correlate the enzyme activity with various pathological conditions. . . . The mass of data obtained, however, has not allowed of any conclusions as to the relation between ChE activity and the disease investigated."

Reduced serum cholinesterase values have been found in liver disease by a number of workers (McArdle, 1940). Hutchinson *et al.* (1951) have recently shown that the plasma enzyme is very significantly lowered in malnutrition. There is little firm evidence to indicate any great variation during disease in the amount of cholinesterase in red cells.

Summary

The suitability or otherwise of the various common methods for the routine estimation of cholinesterase is described.

It is suggested that the "cholinesterase number" (ChE No.) should be determined as a routine screening test, using the Michel electrometric method for the purpose, and that any departures from normality should be investigated in a more orthodox manner by determinations of red-cell and plasma cholinesterase, also using the electrometric method.

Details of techniques for obtaining blood samples and a description of the reagents, apparatus, and methods of performing the enzyme analysis are given.

The problem of normal values of the ChE No., together with the population and personal variation of both enzymes, is discussed. Values of normal limits are included.

In only two pathological conditions—liver disease and malnutrition—is there firm evidence that the level of serum or plasma cholinesterase is lowered. There is no clear-cut evidence that the level of the red-cell enzyme is seriously influenced by disease.

Acknowledgment is made by one of us (D. R. D.) to the Chief Scientist, Ministry of Supply, for permission to publish this paper.

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CARCINOMA OF THE OESOPHAGUS

THE DIAGNOSIS AND SURGICAL TREATMENT OF 159 CASES

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This paper presents the clinical aspects and pathology of 159 patients suffering from carcinoma of the oesophagus who were admitted to Hammersmith Hospital during the past nine years. We have reviewed the surgical results during the period 1946–9 inclusive. The late Professor Grey Turner, who played such a great part in laying the foundation of oesophageal surgery, was in charge of the cases before 1946, and since then one of us (R. H. F.) has continued with the management of this condition.

Major discoveries and minor modifications during the past few years have resulted in a steady improvement in the surgical results, and attention is drawn to those adjuncts that have been of particular help.

Clinical Aspects

Age and Sex.—Of the 159 patients, 72% were men and 28% women. The average age of the men was 63 years and of the women 64. The youngest patient was a man, aged 22, with a resectable growth of the upper third of the oesophagus. The oldest patient was a woman, aged 81, with an inoperable growth situated in the middle third of the