Nevertheless it must be admitted that medical evidence has been of little assistance to the courts, for the obvious reasons that no reliable large-scale data have been available, and the experience of any single witness can provide no clear evidence. For no obstetrician, however active, can expect to see many very long periods of gestation during his working life, and those which he does see provide no basis for assessment of their frequency. In these circumstances it is not surprising that judges have preferred to rely on their own interpretation of common opinion.

The position of a medical witness consulted on this question is on all fours with that of a demographer invited to express an opinion about the longest possible duration of life. He can give a period of gestation which would generally be accepted as impossible, by quoting a figure well outside his own and everyone else's experience. He can give the longest period of gestation for which he has reasonably reliable evidence, whether from his own experience or from the medical literature, and may even be able to state the frequency with which it has been observed in a population of births. What he cannot do, and cannot be expected to do, however full the information at his disposal, is to state a precise figure below which a period of gestation is possible, and above which it is impossible. The translation of medical probabilities into legal certainties is a matter for the courts.

In attempting to provide evidence which may assist the courts we have recorded the period of amenorrhoea from onset of menstruation to birth, and have estimated the period from coitus to birth by allowing for the probable minimum interval between onset of menstruation and coitus. This is the only procedure which lends itself to investigation of the large series of births needed to establish the range of the period of gestation, since dates of coitus, ovulation, and fertilization are usually unknown. Recent legal discussion of the possible interval between coitus and fertilization is indeed irrelevant, for this interval would be of interest only if medical evidence could establish accurately the range of the period between fertilization and birth. In fact, medical evidence is no more reliable on this point than on the period between coitus and birth.

Without recapitulation of reservations expressed earlier we may summarize experience of these 15,669 births as follows: the longest period of gestation which could be accepted with fair confidence is 328 days; the longest period which could not be regarded as impossible is 354 days.

Summary

The period of amenorrhoea from onset of the last menstrual period before pregnancy to birth was estimated for 15,659 of the 18,819 Birmingham births delivered in 1950. In 247 cases the period was longer than 44 weeks (308 days) and in 7 cases longer than 48 weeks (336 days). The two longest periods were 356 and 359 days.

Of the 247 births in which the period of amenorrhoea was longer than 44 weeks, 137 were subsequently excluded, either because the date of the last menstrual period was considered unreliable or because there was a history of bleeding in the early months of pregnancy. Of the 110 periods considered reliable the two longest were 339 and 359 days.

The period between coitus and birth was assessed by subtracting from the estimated period of amenorrhoea the probable minimum interval between the onset of the last menstrual period and coitus. Before making this adjustment it was considered necessary to exclude 35 cases (of the 110 judged reliable) in which the interval between menstruation and coitus could not be assessed accurately because the mother stated that her menstrual cycle was irregular or prolonged in the six months before pregnancy began.

For the remaining 75 cases in which (a) the estimate of the period of amenorrhoea was reliable and more than 44 weeks, and (b) regular cycles of approximately 28 days preceded pregnancy, the minimum interval between onset of menstruation and coitus which might result in fertilization was taken as five days. The longest estimates of the period of gestation (coitus to birth) which could be accepted with fair confidence were then 319, 320, 321, 325, and 328 days. The possibility of periods of gestation longer than these is discussed.

We are much indebted to Dr. Enid Charles for making available to us the distribution of Birmingham births in 1950, and to Mrs. M. Hopper, who interviewed mothers whose period of amenorrhoea was longer than 44 weeks. We are also grateful to the staffs of Birmingham hospitals and welfare centres for permitting us to use their records.

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OESOPHAGITIS*

BY

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You are no doubt aware that at the moment a great interest is being taken in inflammation and chronic ulcer of the lower oesophagus, and that in a sense they are new. In another sense they are not, for many of the things we are now considering have been described before. For instance, patients have always complained of their symptoms, though doctors may have misinterpreted them or brushed them aside because they did not recognize the pattern. Pathologists have described chronic ulcers from time to time for over a hundred years. Radiologists have been examining the lower oesophagus for forty years. Endoscopy is even older, having been first introduced eighty years ago, though it is the recent increase in its use that has contributed most to our present theme.

We are not, therefore, dealing with a brand-new discovery of which the profession has no previous knowledge. In such a case we would have no prejudices, and learning about it would be the relatively simple process of absorbing facts. But the lower oesophagus is ground that has been worked over many times before. Everyone knows something about it. In the last decade new facts have been contributed, but mostly we have been re-examining old evidence and looking at familiar things in a different way. This is a complicated process which people move through at different speeds. Some are apt to cling to the views they have held and found comfortable. Others delight in rearranging the counters of our knowledge without necessarily adding to them. At the moment we are in a period of considerable confusion. Old beliefs have been challenged. New ones have been offered but have not yet taken their place. No doubt all this will soon get stabilized in an orthodox opinion, but in the meantime an interim report may be

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valuable even if it will soon be wrong. This sorting out of ideas has great practical importance, even though it may not result in dramatic cures and saving of life. Confusion breeds uncertain diagnosis, and a patient without a firm diagnosis is often worse off than without a cure.

Symptoms

The symptoms of oesophagitis are pain in the lower chest and heartburn. The chest pain is central and is described as being like wind, food stuck, or a pressing or burning sensation. When it is more severe it radiates to the left side and back. When it is severe, and especially when it is chronic, there is a constant feeling as if someone's knuckles were pressed into the back of the left lower chest. Usually it has no clear-cut relationship to feeding, though there is often enough to make the patient think it is a dyspepsia. Occasionally it quickly follows the taking of food, and then presents as a dysphagia. Sometimes dysphagia is severe and there may be obstruction. The pain lasts for periods of about half an hour. It does not show periodicity over weeks or months like a peptic ulcer.

Heartburn is a lay term, and is used by patients to describe various symptoms. I think most doctors would expect it to mean a burning or hot pain that rises from the upper chest into the throat and may spread into the jaws and mouth and be accompanied by flushing of the face and watering of the eyes. It feels as if some acrid fluid is being forced up the gullet, and the patient swallows repeatedly to keep this down. Regurgitation may indeed occur, but it is not common, and regurgitation is not necessarily accompanied by heartburn. It is most characteristic in this syndrome when it is made worse by stooping. Patients give up scrubbing floors, gardening, or putting, and dread the time they go to bed. Heartburn and chest pain may occur separately or together, and it is difficult to say where one begins and the other ends. There is no point in trying to make this distinction, for they are both oesophagus pains. Chest pain is felt when the lower part is affected and heartburn when it is the upper. One other common feature may be added, and that is rosacea in women. Several of my patients have been under dermatologists for years, and its association with indigestion is of course well known.

These symptoms are as stereotyped as those of peptic ulcer, and one finds patients describing them again and again, once one's ear is attuned to recognizing them. But they are not generally recognized, and we may well inquire why this is so. I think there are two main reasons. The first is that they are the well-known accompaniments of other obvious disease in whose symptomatology they lie buried, or else they are only a minor nuisance and are not taken seriously by patient or doctor. They are, for example, very common in peptic ulcer patients. They are so common with cholecystitis that they are part of the characteristic syndrome. They may also occur as part of almost any dyspepsia. In their minor forms they have been experienced by most people at odd times. In pregnancy they are usual, and, like sickness and swollen legs, are passed off as one of the things you must expect to get. The second reason I suggest for their lack of recognition as a clinical entity was the difficulty of associating them with any organ, pathological change, or objective sign. Even if the lower oesophagus was suspected, there was until recently no means of proving it. The change came when surgeons and anaesthetists mastered the technique of operating safely within the thorax, and it was the surgeon's use of the oesophagoscope that taught the physician to understand what his patients were complaining about.

The Disease

I will now give a dogmatic account of the disease oesophagitis. This account contains much that is believed to be true and a good deal that needs to be proved. The criticism can come later.

The first event is the repeated bathing of the lower end of the oesophagus with gastric juice. This is normally prevented by some mechanism the nature of which is disputed. This is discussed below. In the meantime we may notice that the mechanism—whatever it may be—is liable to be upset when there is any change in the anatomy of the approaches to the stomach. It then becomes incompetent. For example, the oesophagus may be congenitally short, so that part of the stomach lies in the thorax. More commonly there is a hiatus hernia, with a portion of the stomach intermittently or permanently pushed into the chest. Hiatus herniae may also be produced by large abdominal tumours, of which the uterus in the later months of pregnancy is the most notable. The approaches may be interfered with by surgery or destroyed by a growth. Lastly, in a few cases it can be shown that the lower end of the muscular oesophagus is lined by gastric epithelium which may therefore lie above the closure mechanism.

These anatomical abnormalities are not always accompanied by functional incompetence. Nor, on the other hand, is incompetence always due to them. In those cases in which incompetence exists without them, it is presumed that there is some reflex or intrinsic disturbance. This is held to account for the symptoms associated with cholecystitis and early pregnancy, and cases in which barium can be seen to flow back from the stomach with changes in posture.

The effect of constant bathing with acid pepsin is to inflame the gullet, first at the lower end. This is shown by the fact that subsequent regurgitations of acid become painful, whereas normally they are painless. In the early stages nothing abnormal can be seen with the oesophagoscope, and this state may be compared with the soreness that can be felt in a tongue long before reddening can be seen. Later the normal bluish-pink ground, crossed by the tracery of small veins, is replaced by a uniform velvety scarlet which bleeds easily when touched. Then white patches of desquamating epithelium appear. These are followed by superficial oval ulcers with a white or yellow slough surrounded by a red line of inflammation. Intermittent spasm while feeding may sometimes be seen radiologically at this stage.

The erosions heal and recur and nearly always remain superficial. Occasionally one will penetrate deeper and become a chronic ulcer. It is accompanied by much inflammatory oedema, which gradually becomes organized. The thickening is obvious enough at operation or post-mortem examination. At this stage radiological changes become more striking, and it is easy to see the thin trickle of barium through the stricture and the crater projecting sideways like an ulcer in the stomach. These ulcers are often not visible through the oesophagoscope, or only the upper end is seen, because they are buried in the stricture. This accounts for the difficulty there may be in deciding whether the lesion is benign or malignant, or whether there is an ulcer at all. Above it there will be seen the usual inflamed segment with superficial erosions running down between the mucosal folds into the narrow part.

The final outcome is a fibrous stricture. The chronic ulcer may heal and the inflammation above it disappear. It is seen as a smooth funnel both radiologically and with the oesophagoscope.

Prognosis

The outlook when the disease is due to pregnancy, cholecystitis, or cancer is largely determined by cause. In those cases due to primary disturbance of the mechanism it is as follows. The majority of patients who have symptoms and demonstrable reflux get no further complications. It is true that the symptoms may last intermittently over many years or even for the rest of the patient's life. Apart from occasional partial remissions there is little tendency for them to stop. They may get worse, usually gradually, but do not often get better. Those who get a subacute or chronic ulcer with severe painful dysphagia or an obstruct-

ing stricture are very few indeed. It is a possible outcome, but so unlikely that it need hardly be taken into consideration when assessing the patient's future. That does not mean that fully developed cases will not be met with. It means that they are usually seen when already in the later stages. If patients came earlier to their doctors and the oesophagitis were recognized, then more of them would be observed acquiring the later complications. On the other hand, one may be surprised to see a patient with an advanced stricture who has a history of only slight symptoms lasting less than a year. A similar lack of correspondence between the severity of symptoms and maturity of the lesion is seen often enough in ulcers of the stomach.

Criticism

This account now needs modifying. In the first place how certain is it that acid pepsin causes the inflammation? We should be on our guard against a too easy assumption here, for, although it is often held to be the cause of ulcers and inflammation in the stomach, duodenum, and parts of the intestine that may be anastomosed to the stomach, there are still difficulties in this simple view. This question cannot be discussed here, and it is sufficient to point out that we have not got a firm pathology of the ulcerating effects of acid pepsin that could be applied directly to the lower end of the oesophagus. That it has an important role few would doubt, for the frequency with which reflux is accompanied by inflammation is very suggestive and treatment that prevents it is successful. Nevertheless, reflux can occur without inflammation. Many people regurgitate regularly without symptoms, and hiatus herniae are quite common without any disability. Some infants get penetrating ulcers in a remarkably short time, but most regurgitate happily without them. As with peptic ulcer in the stomach and duodenum, we are forced to the same conclusion—that even if acid pepsin is a factor the local state of the epithelium is equally important. There must be some other factor here which prevents inflammation, or fails to prevent it with the same conditions of peptic activity. Despite much research there is no clear understanding of its nature in the stomach and duodenum, and, so far as the oesophagus is concerned, we have got no further than seeing that such a factor is a necessary logical postulate.

This leaves us in the rather unsatisfactory position that the only factor which we know about is the acid pepsin, so that when symptoms are heard or inflammation is seen we look hard with a fixed determination to see what we ought to. The old view of gastric rests in the oesophagus is an example of forcing things too far. When no short oesophagus or the like could be shown, it was held that it was these rests which produced the secretion to inflame the surrounding mucosa, or themselves ulcerated, as they do in a Meckel's diverticulum. It has now been shown that, although such rests are common, they are minute in size and hardly seem adequate to cause trouble, quite apart from the fact that they mostly occur in the upper end. A modern tendency, also resulting from preoccupation with acid pepsin, is to exhort the radiologist to produce some reflux by tipping, stooping, and abdominal pressure. Often he can, but I think it can still be questioned how much bearing these manœuvres have on the normal life of the patient. Perhaps we demand too much of ourselves in this respect, for oesophagitis is a chronic condition which may often develop slowly from intermittent irritation. Even if acid pepsin is the irritant we should not necessarily expect always to demonstrate it on the few occasions that we can examine the patient.

We are surely on firmer ground when we say that acid is the commonest irritant that precipitates pain once oesophagitis is established. The effect of posture is very suggestive. Also the ulceration that may occur with cardiospasm, but which acid never reaches, is never painful. But acid regurgitation often occurs from time to time without these pains. The most obvious example is in vomiting. We must therefore suppose that the inflammation sensitizes the pain

nerve endings so that the normally innocuous acid becomes a painful stimulus. This would be called tenderness in an accessible structure, and we see the same thing with other viscera—when, for example, normal urine becomes an irritant in urethritis. In some cases, especially when inflammation is severe, patients will complain of continuous pain, and it is difficult to believe that their gullets are subjected to a continuous bath of acid. It seems more likely that, as with other parts of the body, a certain degree of inflammation is itself enough to stimulate the pain nerve endings without any adventitious irritant. It is somewhat surprising that the mechanical stimulus of swallowed food is usually painless, for it is only in the most severe and chronic cases, in which there is a chronic ulcer and inflammatory narrowing, that a painful dyspepsia is at all common.

Even if it be granted that these are the ways that pain is caused in oesophagitis, one must not assume that all similar pains are due to the same cause. Heartburn, for example, can occur with achlorhydria; swallowed corrosives feel the same; and in other cases spasm may play a part. The identity of the sensation with various stimuli can be shown experimentally—the pain feels the same whether it is caused by faradic shocks, inflated balloons, acids, alkalis, or neutral irritants.

Some Speculations

A question now arises. Should the physician, when he hears these symptoms, have a mental picture of a red inflamed mucosa? Unfortunately it cannot be definitely answered, though probably he should. Although oesophagoscopy is 80 years old, it was for long a dangerous and uncommon examination. Even to-day, with the great increase in its use, it is mostly employed by surgeons for presumed surgical conditions. The exceptions are the American oesophagoscopists, of whom Chevalier Jackson is the chief. In 1929 he stated: "When the time shall come when every patient with the slightest discomfort or abnormality in swallowing, every patient with pain or discomfort back of the sternum, every patient with gastric haematemesis, every patient with regurgitation, 'heartburn' or 'water brash' is examined oesophagoscopically, peptic ulcer in the oesophagus will be found less rare than it is now thought to be, though it is probably not a common disease."

We may add: "What would we learn about simple oesophagitis?" This day has still not come after 20 years, and it may be argued that it never should. In the meantime we cannot tell how common oesophagitis is, how often it exists without symptoms, or how often symptoms mean oesophagitis.

These speculations are of considerable importance, for what the physician tries to do depends on his mental picture of the pathology. The fairly recent change in attitude to gastric and duodenal pains is a case in point. In the days when he believed pain was caused by hyperacidity, hypacidity, and so on, his diagnostic tests were concerned with studying changes in the juice, and his rational therapeutics were chemical and surgical procedures that altered it. But when the ulcer came into its own again our attitude changed. Our diagnosis now consists in the anatomical demonstration of a deformity, and our treatment tends more and more to its surgical removal.

We will now pass from this discussion of how gastric juice inflames the oesophagus to consider how it gets there. This is quite the most confused part of the subject. Partly this confusion is due to what has been thought in the past and which still remains with us as a legacy. I had hoped to put these in proper perspective by considering the history of their development in detail, but this is unprofitable. I will content myself with two observations, which I hope will clear up some misapprehensions. The first concerns the short oesophagus. It was originally held that this was always a congenital failure of descent. The cases were studied *post mortem* and were advanced. Later, when

radiologists began to show that the stomach could often be seen in the thorax, it was supposed that the cause was still the congenital shortening. It is not denied that such cases do exist, but they are now held to be uncommon. It is agreed that the fault in most cases in which the stomach is seen in the thorax is a hernia of the diaphragm.

The second point is the question of scar contraction of the oesophagus. During the war, when thoracic surgeons began to deal with advanced cases, some were seen in which the oesophagus appeared to shorten. This gave rise to the view that the stomach was drawn up by scar contraction of the oesophagus. This appearance was in fact due to an extension upwards of the stricture, and I think it is now generally agreed that scarring does nothing but narrow and fix the oesophagus.

The Question of Reflux

We come back, then, to the normal mechanism that prevents reflux, and it would be logical in discussing its pathology to describe how it normally works. Unfortunately this is not fully understood. There are three hypotheses current. The first is that the right crus of the diaphragm acts as a pinch-cock, either by squeezing the gullet or by pulling it downwards and backwards. This hypothesis is supported by the fact that herniation is often accompanied by incompetence, and fixing the stomach back into the abdomen by cure. On the other hand, hiatus herniae are not always incompetent, and it cannot be convincingly shown that the point of radiological closure corresponds with the crus. In any case, there is normally squamous mucosa in the short part of the oesophagus in the abdomen, lying below the pinch-cock. Lastly, we do not find reflux when the diaphragm is paralysed.

The second hypothesis is that the oblique entry of the oesophagus into the stomach, together with the arrangement of the intrinsic muscular fibres, constitutes a valve. This is an old view based on anatomical studies, and it is difficult to show that it really works. It would account for the failures of the pinch-cock hypothesis, but does not easily explain its successes. The last hypothesis is the suggestion that neither of these is satisfactory, and that there may, after all, be a functional sphincter at the lower end of the oesophagus, even though it cannot be demonstrated anatomically. The field, in fact, is open, and these remarks have been made not to show how the mechanism works but to point out that there is no answer to be had from asking. It is a subject that does not perhaps directly concern the physician, but it is of the first importance to the surgeon who is called upon to make a repair.

Clinical

Now let us leave this discussion and consider what our attitude may be when confronted with a case. Whether these views will be found to be right or wrong, we have to manage our patients with the knowledge at our disposal.

There is always a tendency, in the enthusiasm of recognizing something new, that one may concentrate attention on it to the exclusion of what is old and humdrum. As a matter of self-discipline, then, there are three things to be remembered. First, that these symptoms are often secondary to pyloric ulcer, cholecystitis, or cancer, and that these must be excluded. Secondly, that the chance finding of a hiatus hernia, short oesophagus, or the like is an abnormality to be left undisturbed unless it is producing symptoms. Finally, the symptoms must be the right ones if they are to be attributed to the abnormality found. Patients complain of so many unclassifiable pains in the belly that the physician is apt to grasp at any straw that floats by. This is harmless enough if it merely consists in giving a diagnosis that the next generation will reject. But when he holds an anatomical abnormality responsible for symptoms we inevitably start putting it right by surgery. This may lead to meddling, as the history of appendicectomy, visceropexy, and much of gynaecology shows.

Routine radiology will show the grosser abnormalities of hernia, reflux, stricture, and spasm. Spasm may be diagnosed as cardiospasm; but this is sometimes a dangerous diagnosis to make, especially if there is pain, for the spasm may be due to an inflammatory or a neoplastic lesion. Stricture is clearly seen, and often there may be an ulcer crater as well. In such cases it is not always easy to be certain, even with oesophagoscopy, that there is no cancer, and exploration through the chest may be necessary. What to do when there are no radiological changes is more difficult to decide, and it depends on the circumstances of the case. If there are mild symptoms a diagnosis can be made on these alone. Often there is a background of uncertainty and of conflicting diagnoses having been made, and in these it is essential to get some support for one's suggestion. An oesophagoscopy is final if inflammation is seen, though it is not yet certain how often this will be found as the milder cases are examined. When the symptoms are severe enough to warrant surgical treatment the emphasis is on finding an anatomical abnormality. It will of course be helpful to have seen inflammation through the oesophagoscope, for this confirms the diagnosis and shows that the trouble has advanced to this degree. But, since the only radical cure is the repair of the mechanism, the most important thing is to demonstrate radiologically that it is faulty. It is in these cases that the radiologist may be encouraged to produce a hernia or reflux by stooping and compression, and the examination may be justifiably repeated, for these things do not happen every time.

Treatment

The most effective treatment is surgery, and the first duty of the physician is to decide whether this is justifiable. Except in the case of stricture or penetrating ulcer, this decision should be based entirely on the severity of the symptoms. Although we believe that strictures and ulcer are the sequelae of simple oesophagitis, this is by no means certain. In any case, it is a rare complication and much less likely to affect the patient than are the natural hazards of surgery. The decision will be affected to some extent by the kind of repair that the surgeon may be expected to do. Thus it will be easy and sure when a hiatus hernia is found. It will be less sure when reflux is in doubt, and less easy when the oesophagus is congenitally short.

In most cases it will be enough to let the patients know what they have and that it will cause no harm. They may also be comforted by the knowledge that there is always surgery to fall back on if life should become too miserable. Simple measures such as taking small feeds, small amounts of alkali, and sleeping upright are sensible, but, excepting the last, do not make much difference. I do not know that anyone has tried repeated applications of astringents through the oesophagoscope since Chevalier Jackson recommended it 20 years ago.

Surgery in the case of oesophagitis from hiatus hernia consists in fixing the stomach below the diaphragm. In most cases it is successful. Stricture if untreated is a fatal condition. Dilatation with bougies will temporarily make swallowing possible, but it has two overriding drawbacks. The first is that the dilated stricture remains open so that further reflux occurs and the whole cycle of inflammation, ulceration, and stenosis is repeated. The other is that sooner or later a false passage will be made, so that, as Charcot said, "They that live by the bougie will die by the bougie."

Stricture and penetrating ulcer require surgical removal. The lower end of the oesophagus is now well within the limits of practical surgery, though the middle reaches are still dangerous ground even with the expert. If the oesophagus can be drawn down and anastomosed to the stomach below the diaphragm, then reflux may not recur. If it cannot, the stomach must not be drawn up into the thorax, for this again leaves the patient with the stage set for return of the trouble. Various devices are being tried to avoid

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