immediately lethal. Dr. Dudgeon and I are further testing the possible conveyance of mouse cancer, by spraying the unkillled cells, suspended in salt solution, into the air inhaled by mice.

Were cancer ever an airborne disease, however, primary cancers of the lung should be something not uncommon. But its extreme rarity proves that such a contraction of the disease could at the most be merely a curiosity and devoid of any great practical issues. The proclivity of the larynx to carcinoma can be explained without recourse to an airborne theory.

And, lastly, for the practitioner interested in the Mendelian aspect of evolution, the construction of medical pedigrees is, as Brevet-Colonel Firth has pointed out, a piece of work that would be of real value. The problems which concern the heredity of such diseases, as goitre, cystinuria, alkaptonuria, tuberculosis, cancer, and insanity can only be elucidated by the study of critically collected histories obtained in individual families.

The Nervous System in Chronic Alcoholism.

(With Special Plate.)

By F. W. Mort, M.D., F.R.S., F.R.C.P.,
Physician to Charing Cross Hospital and Pathologist to the London County Asylums.

As physician to a large general hospital situated in the middle of the liquor traffic and as Pathologist to the London County Asylums, I have had unusual opportunities of studying the effects of alcohol upon the sane and well fed and the sane and ill fed, as well as upon the insane and potentially insane well fed and ill fed. It is only by a comparative study of the individual that a true estimate of the effects of alcohol upon the nervous system can be properly gauged, and it is desirable to approach the subject in a scientific spirit and without prejudice.

The effects of alcohol are largely dependent upon not only the quantity and quality of the liquor taken and the period of time over which it has been taken, but upon the personality of the individual, and by the personality of the individual I mean the physical and mental characteristics which differentiate him from every other individual.

I will illustrate this point first by showing the photographs of a number of chronic inebriates who are sent to reformatories. I will call your attention to the striking evidence in the physique of these people indicative of feeble-mindedness, and I will correlate this fact with an extract from the report of Dr. Branthwaite for the year 1905, concerning Certified Inebriate Reformatories established under the Inebriates Act, 1879–1900. At page 10, he remarks:

Upwards of 62 per cent. of the persons committed to reformatories under the Act are found to be insane or defective in some degree. I am satisfied that the majority of these inebriates have become alcoholic because of congenital defects or tendency to insanity, not insane as a result of alcoholism, and that the drunkenness which preceded alcoholic insanity was merely the herald—the only obvious sign—of incipient mental disorder. In relation to the final insanity, drunkenness in such cases is the intensifier, perhaps, but not the cause of the disease. These chronic alcoholics who eventually come under the care of the State may be divided into two classes: (a) Defectives, imbeciles, and epileptics; (b) moral and social defectives.

A marked intolerance to the action of alcohol is present in both the refractory and quiet class of defectives; very small quantities of drink, no more than is taken daily without apparent physiological effect by an ordinary individual, being sufficient to cause disorderly and violent behaviour. Our experience in this direction has led us to accept the view that intolerance of the existing effects of small quantities of alcohol should be considered a fairly certain sign of impaired mental equilibrium.

I have long been struck by the fact that whereas cirrhosis of the liver with ascites is relatively common in the wards and post-mortem rooms of the hospital, I have only once seen a case of advanced cirrhosis with ascites in the asylums, and that was in the case of a notorious police-court character who was convicted of drunkenness nearly 400 times before she was found incapable of taking care of herself and certified as insane. I am forced to the conclusion that the effects of alcohol upon the mind depend not only upon the quantity, quality, and period of time alcohol has been taken, but even more upon the personality...
of the individual—his temperament, organic constitution, and the circumstances which led to intertemporaneous poverty, insufficient food, and miserable surroundings. It is probable that a person who can drink to a condition of advanced cirrhosis of the liver has inherited an inborn stable mental organisation. In discussing the question of alcohol and insanity we have to consider whether alcohol is the efficient cause of the mental disease, a coefficient with other bodily and mental causes, or merely a coincidence. If we compare the statistics of hospital and asylum post-mortem examinations, we shall find that the fact is last in this formula. There are a large number of cases of advanced cirrhosis of the liver, whereas in the latter there are relatively few cases of cirrhosis, and many of those recognizable with difficulty. I throw on the subject a statistical analysis prepared for me by my assistant, Dr. J. P. Candler. The results obtained in 1,099 autopsies on adults at Charing Cross Hospital, post-mortem examinations and the results obtained by Drs. Rolleston and Fenton on post-mortem records extending over ten years at St. Catherines Hospital are given, and in the main it supports the opinion that the statistics derived from Charing Cross Hospital are reliable, to which could be added other London hospitals. The principal points of interest which this tabular analysis presents is a comparative inquiry into the post-mortem incidence of cirrhosis of the liver at Charing Cross Hospital and Claybury Asylum. There is a close relationship to the subject of alcohol and insanity may be summarized thus: In discussing the rates of cirrhosis at Charing Cross Hospital the notes of the autopsies upon 1,099 adult cases were examined—735 males and 364 females. Of this number there were 85, or 7.7 per cent., cases of cirrhosis of the liver, which accords closely with the 8 per cent. in which this was the immediate and direct cause of the disease for which the patients were admitted to the hospital. The percentage of males is 9.1 and the females 4.9. In Claybury Asylum the notes of 1,271 autopsies were indexed—1,027 males and 244 females. Of this number only 23 cases of hepatic cirrhosis were found (14 males and 9 females). The total percentage of cirrhosis of the liver works out at 1.8 per cent. (males 2.4 per cent. and females 0.7 per cent.). These are a number of points of interest to which the synopsis refers, but I will limit my remarks thereon to the following more important facts which have been elicited—namely, that only 1 case of cirrhosis with abdominal signs in Charing Cross Hospital cirrhosis cases 66.6 per cent. had accidents, 22.2 per cent. with a history of paraesthesia abdominis. At Charing, in 1 instance of the 23 cases was cirrhosis of the liver mentioned as the assigned cause of death, whereas at the hospital and asylum classification, whereas of the 85 cases cirrhosis of the liver was the assigned cause of death. It was noteworthy that the cases of well marked cirrhosis met with on the post-mortem table at the asylum were large livers, and they occurred in persons who had a well marked history of chronic alcoholism, and who during life presented physical signs and mental symptoms of chronic alcoholic insanity, notably alcoholic dementia and polyneuritic psychoses (Korsakow). There were, in addition, some cases of general paralysis. The relatively greater frequency with which acute and chronic gastritis and other inflammatory lesions of the stomach are met with in cases of alcoholic affections of the liver among the insane is shown.

There is a greater frequency of arterio-sclerotic changes associated with cirrhosis of the liver in the case of the insane, especially among the males. Alcohol is common in the post-mortem table, even in the comparatively young people, in asylums. Probably this may be explained by the fact that a large proportion of the deaths occur in the subjects of general paralysis, and this is in all probability due to the syphilitic origin of the disease.

It may be remarked that in only four of the fatal cases occurring at the hospital neuritic symptoms were associated. In fact, it is noteworthy that alcoholic cirrhosis of the liver with pronounced ascites and a history of prolonged intemperance, even excessive intemperance, frequently occurred in individuals who may exhibit no obvious mental symptoms beyond a weakened will, and loss of moral sense, showing the indulgence of a vicious habit.

Comparative statistics of alcohol as a cause of insanity in persons admitted to the London County Asylums for the last thirteen years show an amount of variability of per cent. of the insane, but it is noteworthy that in a small class of the population, that can be in great part explained by a difference of opinion by medical officers as to the statistical evidences of alcoholism and alcoholism of interest and interest in ascertainment a complete family history. Not only have we, but we are to consider the personal equation of the medical officers who obtain the results, but also the data of the friends who give it, as to what constitutes alcoholic excess and as to how far alcoholism is an efficient cause of the statistics which I place upon the screen derived from the annual reports of the Asylums Committee illustrate these facts. In one year, 1902, from the same class of people, alcohol is assigned as the cause of death in Hanwell, and to Claybury 11.2 per cent.; but in 1905, intemperance is the assigned cause of 26 per cent. of the admissions to Claybury, while at Colney Hatch it is only 14 per cent. At Bexley Asylum, where they have adopted the admission of alcohol, it is stated to be nearly uniform since its opening, intemperance as an assigned cause is very high, the average being 22.8 per cent. for the seven years. An analysis of the cases admitted during 1905 to this asylum in which intemperance was the cause of death shows that 12.5 per cent. of the total admissions, shows that there are many cases in which other causes are associated. Of 248 male admissions, alcoholic excess was the assigned principal cause in 46, or 18.5 per cent., and out of the 248 female admissions alcoholic excess was the principal cause.

**DESCRIPTION OF SPECIAL PLATE.**

Fig. 1.—Section of normal cortex from ascending frontal convolutions, to show the (a) tangential, (b) super-radial, (c) inter-radial association systems; the latter intersects at right angles the radial fibres. Magnification 30.

Fig. 2.—Section of the posterior cortical area of the left occipital lobes, showing (a) the tangential, (b) super-radial, (c) inter-radial association systems. Magnification 30.

Fig. 3.—Same section magnified, showing (a) the tangential, (b) super-radial, (c) inter-radial association systems. Magnification 80.

Fig. 4.—Same section as Fig. 2, more highly magnified. Compared with Fig. 2, there is a disappearance of the tangential and superficial systems of fibres. Magnification 80.

Fig. 5.—Section of prefrontal cortex from another case of chronic alcoholic dementia; there was a history of 15 years’ intemperance, and evidence of advanced cirrhosis of the liver (without ascites), vide Fig. 7; it will be observed that the normal aspect is much less marked in the pyramid layer, but there is atrophy and disappearance of the tangential and super-radial systems of fibres. Magnification 80.

Fig. 6.—Section of ascending frontal convolution, for comparison with Fig. 5; observe that there are tangential and super-radial fibres, although there is some degree of atrophy and evidence of active degeneration by the existence of varicosities on the fibres of the tangential system. Magnification 80.

Fig. 7.—Liver showing advanced cirrhosis; the nodular appearance of the surface of the liver. Magnification 50.

Fig. 8.—Small Bet's psycho-motor cell, from the ascending frontal convolution of a case of chronic alcoholic dementia, showing eccentric nucleus and perinuclear eosin. Magnification 1/5.

Fig. 9.—Cells of the vagus nucleus in which degeneration of the vagus nerve was found; from a case of polyneuritis psychosis, acute fatty degeneration of the heart and eosinophilic hamorrhages, Magnification 350.

Fig. 10.—Section of cortex of a rapidly fatal case of polyneuritis psychosis with eosinophilic hamorrhages. There is atrophy of the association systems, for the section was stained by the Weigert method to display the fibres. The black irregular patches are hamorrhages in the cortex. These were visible throughout the central cortex, and extending in some cases to a large part of the head. They are caused by the rupture of small vessels which were in a state of chronic inflammation and fatty degeneration. Magnification 80.
TO ILLUSTRATE DR. F. W. MOTT'S PAPER ON THE NERVOUS SYSTEM IN CHRONIC ALCOHOLISM.
in 38, or 15.4 per cent., a total percentage on the whole admissions of 17 per cent. But when we inquire into these cases we find that 13 were inbociles, 13 were epileptics, 5 were cases of chronic delusional insanity, 5 were of alcoholism, and no less than 20 were cases of primary dementia. In fact, out of the 84 cases, quite one half were lunatics or potential lunatics and the subjects of an inborn tendency to mental disease. I will put on the screen a statistical table to illustrate this. My experience as a clinical observer is that this is the most fruitful cause of pauperism and crime. Sullivan asserts that 60 per cent. of the crimes of violence are due to drink.

The psychoses which occur in the subjects of chronic alcoholism may be divided into three groups, which are the result of the direct or indirect action of alcohol upon usually a previously healthy brain for a considerable period of time—that is, delirium tremens and polyneuritic psychosis (Korsakov's disease).

2. Mental affections resulting from alcoholism occurring in the individual who has always been a non-alcoholic, is said to possess a morbid temperament. At least, this is the explanation I should offer, because the cases in many respects are hardly distinguishable from typical insanity of abstainers.

Inclusive in the period of psychosis is the highest incidence of delirium tremens, and the second highest incidence of delirium tremens and polyneuritic psychosis. Between the psychosis and the alcoholic delirium tremens are the cases of delirium tremens occurring in the chronic alcoholic.

In a few cases of psychic derangement of employees in theシリーズの-winning drinking is not without some cause; and it may be that the work is responsible for the increase in drinking. It has been shown that alcoholism is responsible for a good deal of insanity, and may be responsible for bringing about an incipient general paralysis. Many are admitted on account of threatened or attempted suicide, and a few on account of murder, suicide, and suicide.

Coincidences and causes may thus be confused, for a lapse from control into insensibility may be the first recognizable sign of the mental breakdown. Especially is this the case with general paralysis, and the involuntional psychoses occurring in the climacteric period in women, also men between 50 and 60 who suffer with melancholia, and at times are often the subjects of sclerosis. Again, cases of periodic or manic depressive insanity and dementia praecox may take time to drink. There can be no doubt that neurasthenics, epileptics, imbeciles, degenerates, and potential lunatics possess a marked intolerance to alcohol and the failure to discriminate between what is heredity and what is the result of alcoholism has been the cause of much confusion. Many cases of chronic alcoholism seen in hospital practice are persons who have drunk a large quantity of alcohol every day for a number of years and have come after many operations of paracentesis abdominis, from advanced cirrhosis and ascites. They do not, as a rule, show signs of mental derangement beyond a weakened will, a losing memory, a blurred disease, and not infrequently incipient general paralysis. Many are admitted on account of threatened or attempted suicide, and a few on account of murderers assaults and sexual crimes.

Coincidences and causes may thus be confused, for a lapse from control into insensibility may be the first recognizable sign of the mental breakdown. Especially is this the case with general paralysis, and the involuntional psychoses occurring in the climacteric period in women, also men between 50 and 60 who suffer with melancholia, and at times are often the subjects of sclerosis. Again, cases of periodic or manic depressive insanity and dementia praecox may take time to drink. There can be no doubt that neurasthenics, epileptics, imbeciles, degenerates, and potential lunatics possess a marked intolerance to alcohol and the failure to discriminate between what is heredity and what is the result of alcoholism has been the cause of much confusion. Many cases of chronic alcoholism seen in hospital practice are persons who have drunk a large quantity of alcohol every day for a number of years and have come after many operations of paracentesis abdominis, from advanced cirrhosis and ascites. They do not, as a rule, show signs of mental derangement beyond a weakened will, a losing memory, a blurred disease, and not infrequently incipient general paralysis. Many are admitted on account of threatened or attempted suicide, and a few on account of murderers assaults and sexual crimes.

Coincidences and causes may thus be confused, for a lapse from control into insensibility may be the first recognizable sign of the mental breakdown. Especially is this the case with general paralysis, and the involuntional psychoses occurring in the climacteric period in women, also men between 50 and 60 who suffer with melancholia, and at times are often the subjects of sclerosis. Again, cases of periodic or manic depressive insanity and dementia praecox may take time to drink. There can be no doubt that neurasthenics, epileptics, imbeciles, degenerates, and potential lunatics possess a marked intolerance to alcohol and the failure to discriminate between what is heredity and what is the result of alcoholism has been the cause of much confusion. Many cases of chronic alcoholism seen in hospital practice are persons who have drunk a large quantity of alcohol every day for a number of years and have come after many operations of paracentesis abdominis, from advanced cirrhosis and ascites. They do not, as a rule, show signs of mental derangement beyond a weakened will, a losing memory, a blurred disease, and not infrequently incipient general paralysis. Many are admitted on account of threatened or attempted suicide, and a few on account of murderers assaults and sexual crimes.

Coincidences and causes may thus be confused, for a lapse from control into insensibility may be the first recognizable sign of the mental breakdown. Especially is this the case with general paralysis, and the involuntional psychoses occurring in the climacteric period in women, also men between 50 and 60 who suffer with melancholia, and at times are often the subjects of sclerosis. Again, cases of periodic or manic depressive insanity and dementia praecox may take time to drink. There can be no doubt that neurasthenics, epileptics, imbeciles, degenerates, and potential lunatics possess a marked intolerance to alcohol and the failure to discriminate between what is heredity and what is the result of alcoholism has been the cause of much confusion. Many cases of chronic alcoholism seen in hospital practice are persons who have drunk a large quantity of alcohol every day for a number of years and have come after many operations of paracentesis abdominis, from advanced cirrhosis and ascites. They do not, as a rule, show signs of mental derangement beyond a weakened will, a losing memory, a blurred disease, and not infrequently incipient general paralysis. Many are admitted on account of threatened or attempted suicide, and a few on account of murderers assaults and sexual crimes.
metabolism, do not support this view, but rather we should explain a chronic delusional insanity occurring after alcohol as the result of the effect of the poison upon a subject potentially insane, and who might have developed it if alcohol had not been taken. Before we can solve this or any other problem, it is necessary to have an accurate comparison, clinical as well as pathological, of all the acute and chronic cases of hallucinosis and paranoia in drinkers and abstainers. This might enable us to separate the one from the other. We might ascertain, moreover, that many of the acute cases of hallucinosis, both of which are of a chronic alcoholic psychoses" are not really alcoholic in origin. Another point which, by my mind, requires an investigation, is whether cases of acute hallucinosis occur especially in the subjects of chronic alcoholism, or whether a considerable number develop the habit of drinking after the individual has commenced drinking. I would also suggest the desirability of a complete inquiry into the family history of the different types in order to ascertain the sum total of inherited degenerative tendencies.

In conclusion I would sum up as follows:

The fact that but few cases of advanced cirrhosis of the liver are found in the asylums, and that these occur in cases either of general paralysis, alcoholic dementia, or Korsakow's disease, both of which latter only occur after prolonged intemperance, suggests a priori, that in the great majority of cases of alcoholic insanity alcohol acts as a coefficient to some other factor peculiar to the individual. This hypothesis is supported: (1) By the relatively large number of cases of advanced cirrhosis of the liver dying in the hospitals and presenting no mental symptoms. (2) By the variability of percentages of alcohol as a cause of insanity in the different London asylums as shown by statistics extending over thirteen years. (3) By the precipitation of drunkenness and insanity as shown by comparison of maritime and manufacturing communities with rural communities, this being explicable by the mental and psychical deterioration of the agricultural population in England owing to the migration of the better mental types to industrial centres.

The psychoneuraltic, or Korsakow's disease, was at one time thought to be due to alcohol directly acting on the brain cells. A typical instance of this disease rarely occurs in any other condition than alcoholism; it accords with the later stage of the alcoholic poison and the allied to delirium tremens. It is more prone to occur in individuals with inborn sane mental inheritance than in insane.

The pathogenesis is obscure. The disease, however, is not due to the direct action of alcohol on nervous structures. More probably it is due to deranged metabolism, secondary microbial toxemia, and autotoxemia from denigrated organs which have been injured by the poisonous effects of other alcoholic ingested.

Other causes of this disease are influenza, pulmonary tuberculosis, septic infection, diabetes, and chronic poisoning by some of the metallic poisons, such as arsenic, lead, and mercury.

Drapé states that he has known the disease to be caused by mercurial poisoning in the treatment of syphilis.

This disease occurs more frequently in women than in men, and usually in adult life. An excellent study of it has been made by Ascherson," who states: There are four symptoms which take precedence of all others in importance.

These are: 1. Loss of memory. 2. Paramnesia or pseudo-reminiscence. 3. Mental confusion. 4. Certain peculiarities of the mood.

The loss of memory is for recent events; generally the patients remember the general circumstances of their past, while they forget those which have occurred more recently.

The period of time in the immediate past for the circumstances of which the memory is defective varies in individual cases, but many coincides with that of their drinking.

So far the amnesia is anterograde, and concerns a period during which not only the power of recollection may have been impaired, but when, by reason of the impossibility of getting the material for recollection was probably lacking. In many cases the patients also fail to recollect those circumstances which occurred within a period of time anterior to the period of their disease; the amnesia is retrograde, and, in this case, since the attention of the disease sets in without the power of recollection alone. Clinical observation shows also that the defect of memory is so marked—i.e., that it is of facts, can no longer be forgotten in measure as they are acquired.

French writers have given the name "amnésie continue" to this condition of memory loss, while Korsakow, in his case, has observed in many cases in which the memory is otherwise unaffected. This continuous failure of recollection affects all recollections, and even the patients fail to reproduce ordinary test words and combinations of figures, but they entirely forget the faces they have just seen and the actions of everyday life they have just performed.

Paramnesia (pseudo-reminiscence, false memory), or the representation before consciousness of false experience, is an essential feature of Korsakow's disease. We have recorded the few cases in which occurred with the disease, which are present in cases in which the memory is otherwise unaffected. But one condition that would be difficult to ascertain the cause of is the ideation is limited to reminiscences of memories which are not in the possession of the individual. This has not been observed, but the ideal of the memory is present at the time of waking, and the confusion of identity of persons. These isolated symptoms, and, in fact, mental confusion in general, seem to me to denote a condition of postural stupor.

The Mood of the Patients.—Unless frequent attempts are made to hold their attention the patients are more or less oblivious to surroundings and circumstances, and this is the condition of apathy to which their dull and listless facial expression bears witness. When roused they are apt to be extremely irritable, and this is a useful attribute in the recognition of their confused state of mind, although it is not due to some extent to the instability of the emotions.

In severe cases the patient may develop a stuporose dementia and pass urine and feces in the bed. Some patients are inclined to be peculiar, and I have rarely seen an instance of the disease in which it was absent. This symptom has been carefully studied by Korsakow, and has formed the exclusive subject of one of his monographs. Professors Kruepelin and Wehrung have also attempted to explain it psychologically.

Mental Confusion.—A varying degree of mental confusion is always a symptom at some stage of the malady. In some cases it amounts to pure incomprehension, and so manifest a feature as to override the idea of the loss of memory. Such cases were recognized by Korsakow, who spoke of the patient's condition as "dementia presenile," and the early case also the disease of the French writers. Very often the disturbance of the ideation is limited to reminiscences with time and place, with reminiscences of identity of persons. These isolated symptoms, and, in fact, mental confusion in general, seem to me to denote a condition of postural stupor.

The Mood of the Patients.—Unless frequent attempts are made to hold their attention the patients are more or less oblivious to surroundings and circumstances, and this is the condition of apathy to which their dull and listless facial expression bears witness. When roused they are apt to be extremely irritable, and this is a useful attribute in the recognition of their confused state of mind, although it is not due to some extent to the instability of the emotions.

In severe cases the patient may develop a stuporose dementia and pass urine and feces in the bed. Some patients are inclined to be peculiar, and I have rarely seen an instance of the disease in which it was absent. This symptom has been carefully studied by Korsakow, and has formed the exclusive subject of one of his monographs. Professors Kruepelin and Wehrung have also attempted to explain it psychologically.
Physical Symptoms.

The most important physical symptoms are multiple neuritis, and the neuritis is one of the entire nervous system; the nerves of the lower extremities are most often affected, but the upper limbs—especially where occupation leads to their continuous use—are not infrequently affected in them. I have seen the face and even the entire body partially paralysed or paretic, and in two fatal cases of heart failure and fatty degeneration, the vagus nerves and their medullary nuclei (vide Fig. 9) were the seat of characteristic changes. As a result of the neuritis there is a tendency of wasting of muscles, with absence of deep tendon reflexes and more or less reaction of degeneration. The superficial and deep sensory nerves are usually affected; probably the tenderness on deep pressure is due to the chronic inflammatory affection of the deep sensory nerves to muscle. The affection of the skin sensory nerves is shown clinically by severe pains; in one case the patient believed she was on fire and rushed to the window, and would have jumped out had she not been prevented. The next day bullae appeared on the skin of the legs, and subsequent examination of the nerves showed bullae formation of the skin. Microscopical marked evidences of recent acute degeneration by the Marchi method. Besides hyperaesthesia there may be numbness and formation. There is usually in severe cases, paresthesia and granulated anesthesia and analgesia, which, if marked and persistent, is accompanied by glossy skin.

Various deformities and contractures are liable to occur in the limbs from the effects of the disease, and permanent for the deformity the liability to which is increased by the continued effect of the weight of the bedclothes pressing upon the feet. Tremor is present and sometimes tics, and amongst general symptoms may be mentioned dizziness, emaciation, anaemia, headache, vomiting, and diarrhoea. These symptoms may be serious, and show a general deterioration of the organs and their functions.

II.

The following is a histological account of the structural changes in Korsakow's disease:

The brains are generally of good weight, of good convolutional pattern, and do not, as a rule, show much evidence of thickening of the pia-arachnoid membranes, increase of cerebrospinal fluid, or other obvious signs of cerebral wasting. The ventricles are not granular, but there may be a few ependymal granulations in the lateral side of the fourth ventricle. Microscopical examination shows some wasting of the tangential fibres and subpial glia cells proliferation and splitting, but, as a rule, not marked. The brain systems as a general rule are otherwise well preserved. There is no very marked glia cell proliferation in the cortex, and when sections are stained by the Marchi method, the columns of Meynert are not so much distorted or poorly stained, their apical processes are not curled, and there is no marked coarse change of the cortical neurones. The general pathology is that there is no lympho-glial or plasma cell infiltration of the membranes and pia-arachnoid sheaths.

Are there any morphological changes pathognomonic of the polyneuritic psychosis? In my opinion this clinical syndrome is not peculiar to alcohol. Lead, arsenic, and other toxic conditions produce similar symptoms and similar pathological changes, and it makes me suspect that they are not caused by the direct effect of the alcohol, but are the result rather of microbial toxins, or by intoxication caused by deranged metabolism. I have examined microscopically the brains of a large number of alcoholic cases and several lead cases; in all of these there was a pronounced neuritis there were characteristic changes affecting large psychomotor cells.

The changes in these cells are similar to the changes in the anterior horn cells of the spinal cord, and are very evident; the nucleus is large and clear, it is dislocated to the side, sometimes extruded altogether, there is a marked out of focus. The Nissl granules may be entirely absent or only found at the periphery (Vide Fig. 8).

Sometimes the cytoplasm is vacuolated or shows excess of pigment. I have found in a few cases similar changes of the cells of the nucleus of the motor cell. It may be asserted that these changes in the cerebral and spinal motor neurones indicate a toxic action upon the whole motor effarent path. The changes in the anterior horn cells and the cells of the posterior spin'gal ganglia are, however, similar to those produced in animals by section of the nerve—namely, there is swelling of the posterior spin'gal nerve fibres, and the granules found in the Betz cells of the motor area (Vide Fig. 8). Examination of the sensory path in severe cases often shows profound changes in the posterior spinal ganglion cells and degeneration in the posterior roots and columns of the spinal cord. In one severe case a spinal ganglion was destroyed and only a cavity left. It was the fifth lumbar ganglion and there was glossy skin of the foot on the same side and a tracheal sere on the other.

Owing to fatty changes in the vesal cells haemorrhagic extravasations are very evident; these are sometimes extruded from the veins of the brain, and more or less reaction of degeneration by the Marchi method. Besides hyperaesthesia there may be numbness and formation. There is usually in severe cases, paresthesia and granulated anesthesia and analgesia, which, if marked and persistent, is accompanied by glossy skin.

Various deformities and contractures are liable to occur in the limbs from the effects of the disease, and permanent for the deformity the liability to which is increased by the continued effect of the weight of the bedclothes pressing upon the feet. Tremor is present and sometimes tics, and amongst general symptoms may be mentioned dizziness, emaciation, anaemia, headache, vomiting, and diarrhoea. These symptoms may be serious, and show a general deterioration of the organs and their functions.

II.

The following is a histological account of the structural changes in Korsakow's disease:

The brains are generally of good weight, of good convolutional pattern, and do not, as a rule, show much evidence of thickening of the pia-arachnoid membranes, increase of cerebrospinal fluid, or other obvious signs of cerebral wasting. The ventricles are not granular, but there may be a few ependymal granulations in the lateral side of the fourth ventricle. Microscopical examination shows some wasting of the tangential fibres and subpial glia cells proliferation and splitting, but, as a rule, not marked. The brain systems as a general rule are otherwise well preserved. There is no very marked glia cell proliferation in the cortex, and when sections are stained by the Marchi method, the columns of Meynert are not so much distorted or poorly stained, their apical processes are not curled, and there is no marked coarse change of the cortical neurones. The general pathology is that there is no lympho-glial or plasma cell infiltration of the membranes and pia-arachnoid sheaths.

Are there any morphological changes pathognomonic of the polyneuritic psychosis? In my opinion this clinical syndrome is not peculiar to alcohol. Lead, arsenic, and other toxic conditions produce similar symptoms and similar pathological changes, and it makes me suspect that they are not caused by the direct effect of the alcohol, but are the result rather of microbial toxins, or by intoxication caused by deranged metabolism. I have examined microscopically the brains of a large number of alcoholic cases and several lead cases; in all of these there was a pronounced neuritis there were characteristic changes affecting large psychomotor cells.

The changes in these cells are similar to the changes in the anterior horn cells of the spinal cord, and are very evident; the nucleus is large and clear, it is dislocated to the side, sometimes extruded altogether, there is a marked out of focus. The Nissl granules may be entirely absent or only found at the periphery (Vide Fig. 8).

Sometimes the cytoplasm is vacuolated or shows excess of pigment. I have found in a few cases similar changes of the cells of the nucleus of the motor cell. It may be
complicated. He had found that alcoholics who suffered from nervous symptoms very rarely showed affections of the abdominal viscer.a; on the other hand, patients who were in hospital for visceral diseases usually retained their intelligence unimpaired. It was always difficult to get beer and wine and other strong drinks divided into two classes, the sedentary drinkers and the regular boozers. In two cases of the former class he had been struck by the specificity of desire which assailed the patients, and by the fact that they never had any remorse for their deeds. They were comparable to habitual criminals.

Mr. Shattock (President) asked Sir Clifford Allbutt whether the patients he mentioned would have had that same desire for alcohol if they had never tasted it before. He thought the desire was relatively independent on experience.

Dr. E. S. Pasmore (Oxford) said that in one of the sections shown by Dr. Mott there was evidence of definite brain mischief. In those cases, it seemed to him, treatment such as keeping the patient under control for six months would be of no avail. All chronic alcoholics, while showing no visceral affections, had their minds affected, although this might not be observable; accordingly, they should not be trusted.

Dr. C. McVicar (Dundee) said that it was interesting to note that the syndrome of mental symptoms in alcohol poisoning was similar to that of degeneration, namely, a disorientation as to time and place. The degenerate patients were admitted, not so much because of the alcohol, but because of the mental degeneracy, and re-admissions were associated with the taking of very small quantities of alcohol. The recovery of cases of multiple neuritis with paralysis of the limbs, in association with marked mental changes, was remarkable if the patients were kept under observation for one and a half to two years. Cases of alcoholism showing periodicity were usually impulsive and might be very intellectual.

Dr. Mott, in reply, said that Sir Clifford Allbutt's experience accorded very much with his own. It was the poor depressed man who took alcohol instead of food who suffered so badly and became insane. The convivial drunkards, on the other hand, had a high degree of intelligence; they were chronic boozers for years, then they developed ascites and died; they, however, were able to go on with their work. With respect to Dr. Pasmore's statements about the degeneration of the association fibres, he said that it was extremely difficult to collect such cases. These were probably cases of toxic insanity associated with microbial diseases. It was a toxic psychosis more than anything else. He therefore agreed with Dr. McVicar in keeping these patients under observation. Just as the limbs recovered, so did the mind. He was of opinion that management of limbs and joints should be undertaken early, and that the feet should be protected from the weight of the bedclothes by a cradle. He was very much interested in Dr. McVicar's remarks on the similarity between alcohol poisoning and old age. He agreed with Sullivan, who believed that alcohol was a poison, but that the insanity was due to an inborn tendency. He was glad to hear Allbutt's remarks on the uselessness of rhetoric in such questions, and strongly applauded the suggestion of the collection of medical pedigrees.

THE THERAPEUTIC USE OF ALCOHOL VAPOUR MIXED WITH OXYGEN.

BY W. H. Willcox, M.D., F.R.C.P., F.Sc., F.I.C.,
Physician to Out-patients, St. Mary's Hospital, London, W.;
AND
Professor B. J. Collingwood, M.D.,
Professor of Physiology, the Catholic University School of Medicine,
Dublin.

Introduction.

The value of oxygen alone as a most beneficial remedy in cases of illness where there is respiratory and cardiac embarrassment is generally recognized, and oxygen has been extensively used on account of its good effect in these conditions.

Recently Dr. Leonard Hill has published some very interesting and valuable investigations showing the marked effect which oxygen has in diminishing the fatigue following violent muscular exercise of various kinds. He has also shown the value of oxygen in mountain sickness.

In this paper we wish to call attention to the very remarkable stimulant effect on the heart and circulatory system produced by the inhalation of oxygen and alcohol vapour. When this combination is used, it can be readily demonstrated in cases of cardiac failure that the addition of alcohol vapour to the oxygen administered produces a stimulant effect on the circulatory system much greater than that produced by the breathing of oxygen alone. These are the good effects of the oxygen plus an additional marked stimulant effect on the circulation caused by the contained alcohol vapour.

The combination of oxygen and alcohol vapour was first used in some experiments on animals made by one of us in 1907 (B. J. Collingwood), in which it was found that after an overdose in chloroform anaesthesia the mixture of oxygen and alcohol vapour was a very valuable remedy for those cases in which the cardiac failure had supervened as the result of a toxic dose of chloroform. It was found that the effect of oxygen containing alcohol vapour was greater than that of oxygen alone.

As a result of these experiments, oxygen which had been bubbled through absolute alcohol contained in an ordinary glass bottle was administered by one of us (W. H. Willcox) in several cases of illness in which cardiac failure was a prominent symptom, and it was found that the mixture produced a remarkable stimulant effect on the heart and circulation decidedly greater than that produced by oxygen alone. In some of these cases the administration appeared to have been the cause of the prolongation and saving of life. In cases of pneumonia with cardiac failure the mixture of oxygen and alcohol vapour was found to be a very valuable remedy, and attention was called to this in a discussion on the treatment of pneumonia at the Harveian Society in April, 1908 (W. H. Willcox).

The subject of the paper will be considered under the following headings:

1. Physiological experiments which led to the use of oxygen and alcohol vapour.
2. The apparatus suitable for the clinical administration of the remedy.
3. The chemical investigations which have been made in order to determine the amount of alcohol vapour present in the oxygen under different conditions of administration.
4. The therapeutic effects of oxygen and alcohol vapour.

I.—Physiological Experiments.

Investigations were made into the causation of chloroform apnoea when large doses of chloroform had been administered to cats. It was found that during these investigations that frequently marked chloroform poisoning occurred, and the animals presented all the outward signs of death. Respiration had ceased, the blood pressure had sunk to zero, the eyes were soft, and on opening the thorax the feeblest flicker of auricular contractions was the only suggestion of possible recovery. In other cases the poisoning was not so profound, apnoea and a fall of arterial blood pressure being alone observable.

It was found that the apnoea in many cases could be relieved by the administration of carbon dioxide mixed with oxygen and breathing restored. This remedy, however, did not restore the cardiac failure which had supervened, nor did the administration of oxygen alone bear the desired effect in raising the fallen blood pressure. In these cases, where cardiac failure remained after respiration had recovered, it was found that the administration of oxygen which had been bubbled through absolute alcohol was a very valuable remedy, and in many cases the apnoea quickly rose to normal, and the heart's action was restored. It was found that good results followed the administration of air containing alcohol vapour, and the cardiac failure which was not restored by oxygen or air administration recovered when air containing alcohol vapour was administered.

In the above experiments the oxygen and air mixed respectively with alcohol vapour were administered by means of a bellows attached to a tube in the trachea of the
animal. It was found if the absolute alcohol through which the gas was passed had been exposed to air for some time it lost to some extent its beneficial effect, owing no doubt to absorption of water, and thus to there being a smaller amount of alcohol vapour present in the gas which was passed through the diluted alcohol.

II.—The Apparatus Suitable for the Administration of the Remedy.

The simplest method of administration is to have an ordinary bottle of about 16 fluid ounces capacity, fitted with a rubber cork with two holes bored in it. Through the holes in the cork pass two glass tubes, one acting as the inlet, which reaches nearly to the bottom of the bottle; the other serving as the exit, and passing just through the cork. The glass tubes should be bent at right angles above the cork. The bottle should be about one-sixth full of absolute alcohol. It is convenient to have a metal collar round the neck of the bottle to which is attached a wire hook, so that the wash-bottle may be attached to the bed or other suitable support.

The oxygen cylinder is attached by rubber tubing to the inlet tube of the wash-bottle, and to the exit tube is attached a length of rubber tubing, to the end of which is fixed an ordinary glass funnel, for administering the oxygen and alcohol vapour when held over the mouth and nose of the patient. In an emergency the wash-bottle containing absolute alcohol can be dispensed with, and a pad of wool soaked in absolute alcohol placed in the apex of the glass funnel, which is attached to the rubber tubing connected directly with the oxygen cylinder.

A special convenient form of apparatus has been devised by Dr. Collingwood whereby either oxygen alone or oxygen saturated with alcohol vapour may be administered by turning a tap. It is important that the alcohol used should be absolute and when not in use the apparatus or wash-bottle containing the alcohol should have the inlet and outlet tubes closed by rubber tubing into which plugs fit.

Special facepieces for the administration of the oxygen and alcohol vapours have been used on a few occasions, but these were not found suitable for clinical work, since the patients to whom the remedy was given were extremely ill, and they objected to the facepiece, being apparently under the impression that an anaesthetic was about to be given.

No doubt with a special facepiece, such as that used by Dr. Collingwood, more oxygen and alcohol vapour can be absorbed and probably smaller percentages of alcohol vapour in the oxygen would suffice to produce the therapeutic effect than if the administration is carried out in the ordinary way as described above. Usually the administration of the mixture with a glass funnel suffices to produce a marked therapeutic effect in from three to five minutes.

III.—Chemical Investigations.

Numerous investigations have been made in order to determine:

1. The effect of the inlet tube for the delivery of the oxygen being below or above the surface of the alcohol in the wash-bottle.
2. The influence of the rate of passage of the oxygen when the inlet tube of the wash-bottle is below or above the surface of the alcohol.
3. The effect of changes of temperature on the amount of alcohol vapour in the oxygen.
4. The effect of using other alcoholic liquids than absolute alcohol, such as rectified spirits, brandy, and whisky.

The most convenient method of determining the effect of the position of the inlet tube relative to the surface of the absolute alcohol and the influence of the rate of flow of the oxygen was found to be the estimation of the loss of weight of the wash-bottle under varying conditions, considered, a measured amount of oxygen being passed through in each case. The weighings were, of course, made on a specially delicate balance.

The results are shown in Tables I, II, and III.

From the following conclusions are deduced: If the inlet tube is above the surface of the absolute alcohol, then with a rapid flow of oxygen the gas is not saturated with alcohol vapour; with an ordinary rapid flow of oxygen the gas will be less than two-thirds saturated with alcohol vapour. If the inlet tube is below the surface of the absolute alcohol, then the oxygen will be saturated with alcohol vapour in all cases. With a rapid flow a rather greater amount of alcohol will be present in the exit gas, with a slower current of gas, due, no doubt, to some alcohol spray being carried over in particulate form.

The effect of change of temperature on the amount of alcohol vapour present in the oxygen when the inlet tube dips below the surface of the absolute alcohol is shown in Table IV; which table also shows the amount of alcohol vapour present if rectified spirit, brandy or whisky respectively are placed in the wash-bottle.

The results shown in this table illustrate the marked effect which rise of temperature has on increasing the amount of alcohol vapour in the oxygen. The results also clearly demonstrate the importance of using absolute alcohol, and not a weaker spirit, if appreciable amounts of alcohol vapour are to be contained in the oxygen. Thus at 15° C. with brandy or whisky only about 2.2 per cent. of alcohol vapour is present (0.74 grain per litre); with rectified spirit 3.65 per cent. of alcohol vapour is present (1.16 grains per litre); with absolute alcohol 4.7 per cent. of alcohol vapour is present (1.5 grains of alcohol per litre).

In the above experiment the rectified spirit contained 90 per cent. by volume of absolute alcohol; the brandy contained 47.1 per cent. by volume of absolute alcohol; the whisky contained 52.25 per cent. by volume of absolute alcohol.

For determining with great accuracy the percentages of alcohol vapour present at different temperatures with

<table>
<thead>
<tr>
<th>Table I.—Inlet Tube Half Inch Above Alcohol.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>
different kinds of spirit a combustion method was used the oxygen and alcohol being passed through a red-hot tube containing copper oxide, when the alcohol was converted into carbon dioxide and water. The carbon dioxide produced was absorbed in a weighed soda lime tube, and from the increase in weight of the tube the amount of alcohol vapour present could be calculated with great accuracy.

IV.—The Therapeutic Effects of Oxygen and Alcohol Vapour.

When oxygen which has been passed through absolute alcohol is administered to a patient, there is no doubt as to the remarkable beneficial effect in a suitable case. In many cases of acute illness a period arrives at which life may be said to hang on a thread, and if the patient is able to survive this critical period often complete recovery takes place.

Examples of this are the later stages of pneumonia, the shock following severe operations, especially those on the abdomen, the heart failure which occurs sometimes during chloroform anaesthesia, and many other instances which might be cited. Any remedy which has a powerful stimulating effect on the heart and general system is most potent for good at such a critical period, and oxygen which is saturated with alcohol vapour has appeared to us to be a most valuable remedy. In many cases of desperate illness, in which its effect has been carefully observed, we believe that oxygen passed through absolute alcohol has been the means of saving the patient through the most critical period of his illness, and so of saving life.

It is very interesting to note the amount of alcohol given when administered in vapour form with oxygen. One litre of oxygen when bubbled through absolute alcohol at ordinary temperatures will only contain from 1 to 2 grains by weight of alcohol, and only a portion of this heat be absorbed by the lungs, so that very minute amounts of alcohol are absorbed into the system.

Sufficient attention does not appear to have been paid to the method of administration of remedies by inhalation into the lungs. It is well known that these produce a marked physiological action in extremely minute doses if the administration is by deep inhalation. Examples of this are amyl nitrite, where the absorption of a minute amount of the drug in vapour form produces a most physiological effect.

The action of chloroform vapour and other anaesthetic drugs of this type is such that a relatively minute amount of the drug may cause death when inhaled, whereas much larger quantities could be taken with safety by the mouth. Similarly many other drugs, such as atropine and cocaine, in most minute amounts will, if inhaled into the lungs in the form of a finely-divided spray of their solution, produce a definite physiological effect, which will be as marked as that produced by much larger doses if given by the mouth or hypodermically.

The alcohol which is absorbed when oxygen containing its vapour is inhaled appears to be carried direct from the lungs to the heart, and so produces a very marked physiological effect by the direct action on the heart, even though only minute amounts of the drug are being administered. Probably the small amount of alcohol administered in this way is rapidly decomposed, and so does not produce any harmful effect on the tissues of the bodily organs, such as the liver, kidneys, and nervous system.

The administration of alcohol by inhalation must have few, if any, of the objections which can be raised against the administration of alcohol as a stimulant by the mouth. It is well known that formerly alcohol was regarded as a valuable cardiac stimulant, and that it used to be one of the most largely-used remedies for that purpose. In recent years the pendulum has swung in the opposite direction, and it is the custom now to avoid the administration of alcohol in cases of illness as far as possible, and considerable doubt has been cast upon the supposed action of alcohol as a cardiac stimulant.

It is most important that, while realizing the dangers and ill effects of alcohol under certain conditions of administration, the medical profession should not lose sight of the good effects which it can be proved that alcohol may have in certain conditions. The common use of alcohol in the mixture of alcohol, ether, and chloroform, known as A.C.E. mixture, has, no doubt, arisen and continued because of the less risk of cardiac failure occurring with such a mixture than with pure chloroform. In other words, anaesthetists have acknowledged by their practice in using A.C.E. mixture for so many years their belief that stimulating effects on the heart when its vapour is inhaled.

A very valuable paper on the action of alcohol on the
Alcohol Vapour Mixed with Oxygen.


After a very careful series of experiments, Dr. Dixon proved that in moderate doses alcohol increased the activity and output of the heart, and that in cases of cardiac failure the rise of blood pressure caused by the administration of alcohol was due mainly to its direct effect on the heart. Dr. Dixon says that alcohol probably produces its effect by providing the heart with an easily assimilable source of energy, supplied to it through an active blood stream kept up and oxidized. Alcohol may thus act as a direct food to the heart.

In considering the action of alcohol, it is most important that the question of dosage and mode of administration should be borne in mind. Every one is agreed that in large doses alcohol has a depressing action on the circulation, and is a very harmful drug to the tissues of the bodily organs generally.

In moderate doses alcohol may be beneficial in suitable cases, and this beneficial action is certainly likely to be most manifest and to be gained without ill effect when alcohol vapour is inhaled into the lungs with oxygen, for here the alcohol is conveyed direct to the heart, and all the alcohol absorbed by the blood passes immediately through the heart. In fast failure the full effect of the drug is obtained on the only organ on which it is known to have a beneficial action. If given in the form of vapour with oxygen at ordinary temperatures, the amount of alcohol administered is so small (1 to 2 grams per litre of oxygen) that it is entirely without an overdose.

It is important to remember that the stimulating effect of alcohol on the heart is shown not on the normal organ, but on the heart which is showing signs of cardiac failure. In moderate doses alcohol has little or no effect on the normal heart, but on the failing heart its effects are marked.

Oxygen containing alcohol vapour has little effect on the normal heart, but a marked effect in cases of cardiac failure. This is because the inhalation of alcohol vapour in oxygen makes the administration of the gas pleasant to the patient, the effect being warming and soothing to the air passages. There is no irritant effect whatever.

In cases of cardiac failure with feeble rapid pulse and cyanosis, the inhalation of the patient of oxygen containing alcohol vapour causes the colour to improve and the pulse to increase in force and volume. If the pulse is rapid—for example, over 100 beats per minute—the inhalation usually causes the rate to be reduced to a运htly usual pulse. In fast failure with slow pulse the administration is often followed by some quickening of the pulse, so that the frequency becomes about normal.

Where the blood pressure has fallen as a result of cardiac failure, it has been observed that the blood pressure quickly rises when oxygen and alcohol vapour are administered, a rise of 5 to 30 mm. of mercury having been measured in several cases with the Riva-Rocci sphygmonomanometer. The patient usually expresses himself as feeling strengthened and refreshed after inhaling the mixture for a few minutes.

It has been found that after the administration of the mixture has been continued for about two minutes the pulse becomes stronger and fuller, and begins to become slower if rapid. It has been found that the full beneficial effect is obtained after about five minutes if a rapid stream of oxygen through absolute alcohol is administered, and if the administration is now stopped the beneficial effect remains or continues for minutes. In cases of cardiac failure it has been found convenient to give the oxygen and alcohol vapour in a rapid stream for minutes every half-hour.

The remedy has been used in a very large number of cases; among these being cases of pneumonia with cardiac failure, cases of heart failure following child-birth, attacks of asthma with heart failure, septicaemia, typhoid fever, myocardial degeneration, dilatation of the heart, and valvular lesions of the heart, collapse after epidemic diarrhoea, etc.

In a large number of cases where oxygen charged with alcohol vapour has been administered and the above described beneficial effects have been observed, namely, the pulse improved in force and volume and slowed, if rapid, together with a definite rise of blood pressure where it has become low from enfeebled cardiac action, it has been found that the administration of oxygen alone without the alcohol vapour has not caused the same improved effect on the pulse and circulation, though, of course, the colour of the patient has improved under the influence of the oxygen when given alone.

So marked have been the results obtained by the administration of oxygen and alcohol vapour in cases at St. Mary's Hospital, London, that it has become the usual practice to keep in every ward of the hospital and in the operating theatres wash-bottles containing absolute alcohol ready for cases of emergency, so that oxygen and alcohol vapour may be administered without delay.

Many medical men have reported to us cases where the administration of the remedy has been followed by marked beneficial results.

For assistance in the above work our thanks are due to Dr. John Webster, F.R.C.S., who gave valuable assistance in the chemical investigations; Dr. E. Holmes, House Physician of St. Mary's Hospital, who made several observations on the effect of the remedy in cases under his care; and to Mr. H. L. Buswell and Mr. R. F. Wilkinson for valuable assistance in the physiological experiments.

Conclusions.

1. The administration of oxygen bubbled through absolute alcohol is a marked cardiac stimulant in cases of heart failure.

2. The administration is pleasant and non-irritating to the patient; it causes no ill effects to the lungs or bodily system.

3. A rapid feeble pulse is slowed by some 10 or 20 beats per minute, and there is a marked improvement in its force and volume. The blood pressure, if low from cardiac failure, is raised from 10 to 30 mm. of mercury as measured by the Riva-Rocci sphygmomanometer.

4. It is better to use absolute alcohol in the wash-bottles, since with less concentrated forms of alcohol an insufficient amount of alcoholic vapour will be contained in the oxygen.

5. The oxygen should be bubbled through the alcohol in a rapid stream.

The full effect of the administration is obtained after five minutes, and lasts for several minutes afterwards. As the effect passes off the administration should be repeated.

References.
3. Dr. Leonard Hill, Oxygen Generator and Inhaler for Use in Mountain sickness, British Medical Journal, November 27th, 1902.
6. Sir CLIFFORD ALLBUTT (Cambridge) asked if the cyanosis disappeared, and also if there had been any experiments with alcohol vapour alone.

Discussion.

Dr. A. E. Boycott (London) said that the amount of alcohol absorbed by the patient must depend on the rapidity of flow of the gases, and that consequently the risk of aspiration would be down expressed inadequately the amount of alcohol taken.

Dr. Mott (London) asked if Dr. Willcox had noticed any alteration in the depth of respiration after the inhalation of the vapour, and whether he thought that the alcohol was acting on the nerves at all.

Sir Clifford Allbutt (Cambridge) asked if the cyanosis disappeared, and also if there had been any experiments with alcohol vapour alone.

Dr. J. Mackie Whyte (Dundee) said that Binz had shown by repeated experiments that the respiratory system was stimulated by alcohol. The explanation of this phenomenon offered by his critics was that it was an effort on the part of the body to neutralize the bad effects of the alcohol, more especially with respect to the interference with the proper oxygen supply of the blood. Possibly the addition of oxygen prevented this bad effect. Could Dr. Willcox say in what way precisely the stimulant
showed itself? Was it on the heart mainly or on the blood vessels—a peripheral vasomotor paralysis?

Dr. Willcocks, in reply, agreed with Dr. Boycott that the figures set down were not high enough; he had not paid particular attention to variations in the depth of respiration. The cyanosis cleared up probably because of the presence of the oxygen. In answer to Dr. Mackie Whyte's remarks, he said that some experiments of Dixon seemed to prove that the action on the heart was more marked.

SOME OBSERVATIONS ON FATAL CASES OF PULMONARY THROMBOSIS.

BY E. R. KNOWLES, M.D., Liverpool.

The fatal occlusion of the pulmonary artery by an embolus or thrombus is deeply interesting, not only to the physician, the surgeon, and the obstetrician, on account of the tragic suddenness with which death occurs, but also to the pathologist who is called upon to decide at the necropsy whether the foreign bodies lodging in the pulmonary artery are the result of embolism, thrombosis, or both. By the majority of writers clots present in the pulmonary artery are invariably ascribed to embolism. There is no mention of primary spontaneous thrombosis in the majority of textbooks on medicine, surgery, or midwifery. A few allude to it as a possibility or of rare occurrence. Embolism is held to be commoner than spontaneous thrombosis by Frederick Roberts, Fowler and Golder, and Welch. On the other hand Playfair, Mery, Newton Pitt, and Box appear to maintain that primary thrombosis is not at all common, but commoner than embolism.

A curious fact is that, though many writers have carefully described the microscopical appearances of the clots found in the pulmonary artery, no attention whatever has been paid to their microscopical structure. This is all the more remarkable since, as is well known, the microscopical structure of a thrombus alters with age. In those cases of supposed pulmonary embolism in which thrombosis extended far into the lungs this extension is assumed to be the result of encasing and secondary thrombosis. But a careful microscopical examination works wonders in demonstrating the truth or falsehood of this assumption.

The following are the changes produced by age in pulmonary thrombus to which we attach the greatest importance:

1. The erythrocytes in a recent thrombus have very distinct outlines, and when stained with cosin are bright red. Later their outlines disappear, they fuse together, and take a brownish tint with cosin. Later still brownish-yellow haemosiderin pigment appears in increasing amount as fine granular deposit, which is soon found inside the cytoplasm of the leucocytes present in the clot. Finally, the surrounding artery wall itself becomes pigmented; the pigment may even be found in adjacent cases.

2. The polymorphonuclear leucocytes, which are present even in aseptic thrombi and tend to increase in numbers through emigration, retain their normal appearance and staining capacity, and are destitute of pigment at first; but soon karyorrhexis and karyolysis of the nuclei take place, the margins of the cytoplasm become indistinct, and the cell eventually disappears. If the leucocyte had contained much pigment its original site is marked by a brown, faintly-granular mass.

3. Organization is proved positive that the thrombus has been in situ for some time. The rapidity with which haemosiderin occurs depends partly upon general conditions, such as the presence of haemolysis in the blood, from streptococci, etc., and probably upon local conditions.

Although we can make no definite statement as to the exact time pigmentation takes to appear, for it must vary with circumstances, it is perfectly clear that the liberation of haemoglobin from the erythrocytes, its deposition as haemosiderin, its entrance into the leucocytes and later into the connective tissue of the surrounding artery, is not a question of minutes, but of hours, sometimes days; and none of our patients lived more than thirty minutes after the onset of the symptoms.

The eight cases about to be described occurred in the post-mortem room of the Liverpool Royal Infirmary during the years 1902-1909 inclusive, and we are indebted to the honorary physicians and surgeons for permission to use them. The thrombi were examined microscopically in all cases. In the last 5 the heart and lungs were removed together, and the distribution of the thrombi was not finally ascertained till the organs had been fixed and hardened.

CASE I.

J. L., male aged 50, admitted 1902, with anterior hypopyon following injury. He declined to have his eye removed, and was kept in bed. On the nineteenth day after admission, on going to stool at 5 a.m., he was suddenly seized with dyspnœa, thoracic pain, and slight cyanosis, and died in about fifteen minutes.

At the post-mortem examination, the right ventricle and pulmonary artery contained no clots, but situated in the stem of the right and left pulmonary artery and extending into the lungs were two branched straightened thrombi, the main stem of each being 5 in. or 4 in. long. The proximal end of one thrombus appeared to be composed of two thrombi adhered together. (The diameter of the thrombii varied from 8 in. to less than 1 in.) The pulmonary artery was empty, so the clots in the right and left branches were separated. Microscopical examination of the collet extremity of the largest clot demonstrated that it was of recent formation, was not composed of two thrombi, but of a single laminated piece, the inner white layers being arranged like a figure of 8. Thus the clots were evidently due to secondary embolism in the thrombosed vessels. Two pieces from the smallest vessels showed extensive pigmentation, and were therefore old. No source for embolism was discovered; the ophthalmic veins contained no clots.

Evidence in Favour of Spontaneous Thrombosis.—(1) Striated thrombi in both pulmonary arteries, separated from one another. (2) Pigmentation of smaller thrombi. (3) No systemic clotting.

CASE II.

E. M., male, aged 12, operated on for appendix abscess, November 26th, 1904. A sinus formed and occasional pyrexia continued until January 8th, when the appendix was removed. The patient was progressing normally, but at breakfast he became cyanosed, and died in less than twenty-five minutes. At the post-mortem examination the abdomen was normal. The heart contained a yellow agony clot, which extended as far as the bifurcation of the pulmonary artery. Beyond this point it became stratified and extended along the larger pulmonary arteries, some branches reaching far into the lungs. Microscopical examination demonstrated that the largest of these, the right inferior pulmonary artery, was an atheromatous clump with a central core of atheroma, the outer surface being split open with a small clot, which extended to a distance of about five inches from the origin of the first branch. No thrombus was found in the right subclavian, axillary, or brachial artery, but there were three clots in the thoracic arteries, the highest of which was in the left subclavian. Microscopical examination showed that these clots were in situ for several months, but had therefore been laid down first. No source of embolism was discovered.

Evidence in Favour of Spontaneous Thrombosis.—(1) Striated thrombi in both pulmonary arteries, also in smaller branches. (2) Pigmentation of smaller thrombi. (3) No systemic clotting.

CASE III.

M. T., female, aged 53, had the right breast and some axillary glands removed for cancer, June, 1905. Progress satisfactory until the sixth day, when the temperature was 101° F., and on the sixteenth day 100° F. At 5 a.m. on the seventeenth day the patient had a cup of tea; at 5.5 it was cyanosed; at 6.1 she was dead.

At the necropsy the wound was healthy, the brain normal. The heart weighed 11 oz., being somewhat hypertrophied and dilated. There was extensive fatty infiltration of the myocardium. A yellow agony clot was found in the right ventricle, and typical laminated thrombi in both branches of the pulmonary artery, extending some distance into the lungs. No thrombus was found in the axillary or iliac vein or inferior vena cava.

No microscopical examination was made of these thrombi, but they were undoubtedly true acute mortems.

CASE IV.

B. D., aged 10, was shot in the hip on July 29th, 1905, at 9 a.m. Suppuration and pyrexia immediately occurred. On August 1st the head of the femur was excised. At 9.45 the next evening the child was fairly well; at 10 p.m. the nurse found her dead.

At the post-mortem examination a red thrombus was present in the right auricle. The pulmonary arteries was empty, so was the right pulmonary artery, even to its terminal ramifications. A laminated thrombus, 0.4 cm. in circumference (0.4 cm.), extended into some of the branches of the upper lobe.
Certain smaller branches also contained clot which was unconnected with the main stem. The right lower lobe contained no thrombi for its greater part, but two vessels in the base with a maximum diameter of 24 mm. were full of clot separate from the main branch at a distance of 4 cm., and spreading into the smallest branches of the artery.

Ten different portions of the thrombi were examined microscopically and demonstrated that the proximal end of the main stem was of recent origin; that its distal end was old, for in the latter pigment had appeared, some being present in the leucocytes, while certain of the smaller thrombi in the lower lobe were older still, because the leucocytes had autolyzed, leaving pigment masses behind them. Occasionally the artery which was also pigmented. Other of the smaller thrombi were of recent origin. The hip-joint contained pus. No source of embolism was discovered.

**Evidence in Favour of Spontaneous Thrombosis.**
1. The presence of pigment in the distal end of the main stratified thrombus.
2. The separation of some peripheral thrombi in both lobes from the main thrombus.
3. The age of some of the peripheral thrombi as indicated by extensive pigmentation.
4. No systemic clothing.
5. The presence of the thrombi in the left lung: embolism is said to be more frequent in the right lung.

**CASE V.**
W. E., male, aged 64, had his jaw excised for epithelium on September 10th, 1927. He progressed normally, but on the 15th his temperature rose to 99.5°. Next day he had a heart attack, and died suddenly in half an hour at 9 a.m. on the seventh day after operation.

**Post-mortem Examination.**—Situated in the pulmonary arteries was a V-shaped thrombus the stem of which measured 10 cm., and was reached into the right ventricle, where it formed an irregular mass about 2 cm. transversely. The limbs of the V spread into both pulmonary arteries, that to the right being separated by an interval from other thrombi which reached into some of the finer ramifications of both lobes, that to the left extended a short way into all the main branches of the upper lobe only, but there were a few isolated thrombi in the smaller branches of the lower lobe. There were some signs of consolidation in the right upper and lower lobes. The systemic veins were not examined.

Ten separate pieces were examined microscopically. The systemic veins were composed mainly of thrombi with some recent thrombus. The right limb showed advanced pigmentation and karyorrhexis of the leucocytes. One smaller thrombus from the right upper lobe was much older; the pigmented part having spread to some adjacent cartilage. The left limb of the V was of more recent origin. Some of the isolated thrombi in the right upper lobe contained pigment which had slightly infiltrated the vessel wall. The pulmonary patch in the right upper lobe contained Gram-negative bacilli about the size of colon bacilli.

Certain veins in the myocardium had evidently thrombosed some days before death, for they were full of pigment. The red blood corpuscles were normal in those arteries which contained blood, and in the majority of the capillaries.

**Evidence in Favour of Spontaneous Thrombosis.**
1. The shape of the thrombus in the pulmonary arteries and the right and left branches, and the absence of encapsulated embolus in it.
2. The isolated thrombi in both lower lobes.
3. The pigmentation of the thrombi and especially of the arterial walls.
4. The spontaneous thrombosis in the myocardial veins.

**CASE VI.**
S. K., aged 46, a woman with a moderately enlarged thyroid and slight exophthalmic symptoms, had hysterectomy for cancer on May 28th, 1936. The operation was followed by slight daily pyrexia. On June 12th and 13th the patient complained of palpitation and uneasiness, but the house-surgeon found the heart normal. On the 14th, at 10 a.m., she became cyanosed, the heart rate rapidly increased, and she died in a quarter of an hour, eighteen days after laparotomy.

On the necropsy the external wound was healthy, but the stump of the left Fallopian tube was necrotic. Commencing at the bifurcation of the inferior vena cava, and extending onwards down the left common iliac and left femoral vein as far as the knee, was a true antemortem thrombus, which was firmly adherent to the vein for 1 in. below Poupart’s ligament. A smaller thrombus extended down the right common iliac vein for a few inches.

The right ventricle contained red and yellow clot, which extended into the pulmonary arteries. Distinct sources of the pulmonary thrombi, by their fibrinization, was a cone-shaped piece of true antemortem thrombus, measuring 2 in. longitudinally, blunt at one end. This thrombus was composed of two vessels, the upper two passing to the upper lobe—one extending into the smaller branches—the third to the lower lobe, almost completely occluding the main vessel, and was adherent to the vessel, the maximum diameter being 1.16 cm.; some of its branches extended into an infarct at the base. A large thrombus extended from the left lower lobe, the artery at its bifurcation to the two lobes, some portions of it extending to the finer branches and others ending blindly. There were small infarcts at both bases.

Fourteen different parts of these thrombi were examined microscopically. The main thrombi in both upper and lower lobes were laminated and pigmented locally. Many leucocytes were also pigmented, but they still stained normally. Some of the smaller thrombi were older, others quite recent. There was pigmentation of some of the artery walls in both lower and the right upper lobe. Two portions of the adherent thrombus in the right lower lobe showed organization, which was more advanced at the periphery where canalization had begun.

A section of the femoral vein with the adherent thrombus showed the clot to be slightly pigmented and organization beginning. There was no round-celled infiltration in the vessel wall and bacteria were absent. Old pigmented thrombi were present in some of the myocardial veins.

**Evidence in Favour of Spontaneous Thrombosis.**
1. The complete separation of the thrombi in the two lungs from one another.
2. The extensive amount of pigmentation in some of the thrombi in every lobe, and specially in some of the vessel walls surrounding them in three lobes, proving that several had been in situ for a considerable period.
3. The organization in the clot of the right lower lobe more advanced towards the periphery.
4. Spontaneous thrombosis in the veins of the myocardium.

**Comment.**
This is the only case in the series with extensive thrombosis of the systemic veins, and it suggests the possibility of embolism, especially as there was a loose thrombus in the pulmonary arteries. But embolism will not explain the peculiar and widespread distribution of the pulmonary clots, their macroscopic appearances such as the absence of signs of fracture, and their microscopical character. It is noteworthy also that organization was not as far advanced nor pigmentation as extensive in the adherent piece of femoral thrombus as in the adherent piece of pulmonary thrombus.

The acceleration of the pulse, which began on the fifth day after the operation—a point which will be alluded to later—also suggests spontaneous thrombosis. The occurrence of clots in both pulmonary arteries separately, in the iliac veins, and in the myocardial veins (other organs not examined microscopically), suggests a general tendency to spontaneous thrombosis throughout the body. The balance of evidence is strongly against embolism.

**CASE VII.**
E. H., male, aged 57, operated upon for inguinal hernia, November 20th, 1936. The operation was followed by slight pyrexia. On the sixteenth day after the operation, while taking breakfast, he complained of faintness. After finishing it and lying down again he was suddenly seized with intense dyspnoea which was slightly cyanosed, and was dead in half a minute, at 7 a.m.

At the post-mortem examination there was slight cellulitis at one end of the inguinal incision. The inferior vena cava, at the site of the left femoral artery at its bifurcation to the two lobes, contained a small piece of thrombus the size of a threepenny-bit, lying over the orifice of the external pudic vein. There was a similar piece in the left femoral vein, in the mouth of the external
circumflex vein. The veins in the calf were normal. The left ventricle was dilated, there being fibroid myocarditis (verified microscopically). No clots were found in the right auricle and ventricle, but a large thrombus began at the posterior cusp of the aorta, into which it partially filled, and extended along the left pulmonary artery into both lobes. Some of the thrombi in the smaller branches were distinct from the main stem. The clots in the left artery was quite separate from that in the right, some of those in the lower lobe being continued into the chest branches. The larger thrombi showed a distinct tendency to coil. There were patches of pneumonia in all, and small infarcts in three lobes.

Fig. 2.—Case VII. Showing separation of thrombi in the two lungs from each other, and separation of individual thrombi in the same lung. (Note the coiled appearance of part of thrombus to right lower lobe.)

Fourteen different pieces of thrombus were examined microscopically. It was found that the large thrombi in the pulmonary arteries and its two branches had been recently deposited, but many of the smaller thrombi were much older, the walls themselves being pigmented in two pieces of tissue from the right, and in three from the left lung respectively. Slender columns of spindle cells, with oval nuclei indicating commencing organization had been in three blocks, one from each lower lobe, and one from the left upper lobe. There were numerous patches of pneumonia, and large colonies of streptococci distributed through both lungs, but none was found in the thrombi, though looked for. The small thrombi from the right femoral vein was pigmented centrally but not organized.

Evidence in Favour of Spontaneous Thrombosis.—
(1) The separation of the thrombi in the two lungs from each other, and separation of individual thrombi in the same lung. (Note the coiled appearance of part of thrombus to right lower lobe.)

(2) Pigmentation of certain thrombi and even of the artery walls surrounding them in both upper and lower lobes.

(3) The occurrence of organization in the left upper and both lower lobes.

CASE VIII.

E. G., female, aged 44, had hysterectomy for fibroids on October 25th, 1909; slight evening pyrexia followed. On the eleventh day a patch of consolidation developed at the right also a loose oval ante-mortem thrombus, 1 in. long, without any signs of fracture in it, and to which was attached a small quantity of red clot; and the right ventricle contained a small quantity of red clot.

Fig. 3.—Case VIII. Showing separation of thrombi in the two lungs from each other, isolated peripheral thrombi in the right lower lobe, and thrombus in vessel base. On the eighteenth day, after a good sleep, patient had a drink, and was immediately seized with an acute precordial pain. She became slightly cyanosed, and died in ten minutes at 4 a.m. At the post-mortem examination the wound was healthy, there was no trace whatever of lymph nor pus. No thrombi were found in any of the systemic veins after a careful search. The right ventricle contained a small quantity of red clot; and the left ventricle was dilated, there being fibroid myocarditis (verified microscopically). No clots were found in the right auricle and ventricle, but a large thrombus began at the posterior cusp of the aorta, into which it partially filled, and extended along the left pulmonary artery into both lobes. Some of the thrombi in the smaller branches were distinct from the main stem. The clots in the left artery was quite separate from that in the right, some of those in the lower lobe being continued into the chest branches. The larger thrombi showed a distinct tendency to coil. There were patches of pneumonia in all, and small infarcts in three lobes.

Twelve different portions of the lungs and clot were examined microscopically. The small thrombi from the left ventricle consisted of laminated material with softening and advanced pigmentation in the centre. The characteristic features of the other thrombi were a slight amount of pigmentation and the commencement of organization in some from both bases, and in an infarct from the left upper lobe. There was some purulent bronchitis in the right base, with clusters of Gram-positive cocci in the lumen of the bronchus and the adjacent pulmonary tissue. This bronchitis probably accounted for the temperature.

Evidence in Favour of Spontaneous Thrombosis.—
(1) The separation of the thrombi in the two lungs from each other, and the occurrence of isolated thrombi in each lung. (2) The occurrence of organization in both lower lobes. (3) The absence of thrombi in the systemic veins. 

Comment.
The large loose thrombus in the right ventricle had apparently been formed in the pulmonary artery, not the heart, because of the absences among the columnae carneae, and the right auricular appendage was empty. Whether it formed in the peripheral veins, because the central softening indicated that the thrombus was a very old one, and it is very improbable, if thrombosis had occurred so long before death in the systemic veins, that no other thrombi should be found in any of them.

General Summary.
The following evidence indicates that the thrombi in the pulmonary artery originated spontaneously in the 8 cases.

A.—Macroscopic.

1. Laminated thrombi were present in both lungs and left lung in all except Case IV, and those in the right and left lung were separate from one another in Cases I, II, VI, VII, and VIII. The shape of the main thrombus in Case V and the absence of central embolus in it has been ascribed to.

2. Peripheral thrombi occurred in the small branches of the pulmonary artery separate from main branch in the larger branches in Cases IV, V, VI, VII, and VIII. The impossibility of laminated thrombi, often extending into the terminal ramifications of the pulmonary artery, being deposited in from one to thirty minutes after the transmission of symptoms following the impaction of the supposed embolus.

B.—Microscopic.

1. Lamination of the majority of the thrombi. 

2. Pigmentation in certain thrombi, usually the peripheral, in all 7 cases examined microscopically.

3. Pigmentation of the artery wall surrounding the thrombi in all 5 cases in which the lungs were examined microscopically—namely, IV, V, VI, VII and VIII.

4. The occurrence of organization of the thrombi in 3 out of the 5 cases in which the lungs were examined microscopically, involving one, two, and three lobes respectively.

C.—Absence of Systemic Thrombosis.

1. No source of emboli was found in the systemic veins in 5 out of 7 cases examined; nor in the heart, including the auricular appendices, in any of the. Through Cases IV and VII contained a loose thrombus in the pulmonary artery and ventricle respectively.

2. The occurrence of pigmented thrombus in the myocardial veins in Cases V and VI, and the small thrombus at the orifice of a vessel opening into each femoral vein in Case VII suggests a general tendency of the blood to thrombose. Even in Case VI, in which extensive systemic thrombosis occurred, the pulmonary clots were probably of spontaneous origin.

Etiology.
This may best be considered in relation to the causes of thrombosis as given by Welch.

A.—Contact of the Blood with Abnormal Surfaces.

There was neither macroscopic nor microscopic evidence of atheromas, arterio-sclerosis, nor acute arteritis in the
pulmonary vessels in any of our cases. The valves of the heart were normal, so was the myocardium, except in Cases III and VII, where the ventricles were dilated from extensive fatty infiltration and showed myocarditis respectively.

II.—Slowing and other Irregularities of the Circulation.
1. All our patients were confined to bed and were consequently exposed to the danger of passive congestion of the lungs.
2. The myocardial disease present in two cases might have interfered with the normal circulation.
3. Thrombosis on the whole was more extensive and older in the lower lobes and bases of the lung. It was also the organization most frequently occurred.
4. With one exception all the patients died in the morning, six before 9 a.m. The physiological slowing of the pulse during sleep probably accelerated the thrombosis.

C.—Bacterial Infection and Chemical Changes in the Blood.
These may be taken together. Bacteria were probably not responsible for thrombosis in all instances, because:
1. Collections of pus were present in Cases I, III, IV, VI, and VII. Case V, with excised jaw, was probably septic.
2. Slight pyrexia usually daily occurred in Cases II, III, IV, VI, VII, and VIII. The temperature chart of Case I is missing.
3. Three out of the five lungs examined microscopically showed acute inflammation and contained clusters of bacteria.
4. All cases had been anaesthetized and operated upon except No. 1, hence the possibility of bacterial infection through the lungs or wound. On the other hand, no bacteria were found in the thrombi or in the walls of the thrombosed vessel, though specially looked for in sections stained with Gram and methylene blue.
It is noteworthy that in half the cases the operation was upon the abdomen; in half also death took place from the fourteenth to sixteenth day after the operation.
The factors described were predisposing, but the determining causes were not apparent.
Assuming that thrombosis was slowly taking place in the pulmonary artery, how is it that no symptoms were apparently produced? The reasons seem to be the following:
1. Gradual obliteration of several branches of the pulmonary artery by thrombosis is far less likely to produce symptoms than the sudden obliteration of a much smaller branch by an embolus.
2. The patients were resting in bed; consequently there was no tax upon their circulatory system.
3. Signs of impending danger may be overlooked in surgical wards. Thus it is not always customary to make a systematic record of the pulse or respiration-rate long after an operation in patients who are apparently progressing favourably; and it was only in the last three cases that we were able to obtain any data.

Here we were pleased to find that the mean of the morning and evening pulse-rate showed a definite and permanent acceleration, beginning from the seventh to the eleventh day after the operation. Thus the average pulse-rate of these patients for the three days immediately following the operation was 86, 86, and 82 respectively, compared with the average pulse-rate for three days immediately preceding death, which was 126, 96, and 106 respectively (see chart).
The charts prove that this acceleration was not due to a rise in temperature. This clinical fact supports the thesis that the patients died from spontaneous thrombosis. Case VII, the only one in which the respirations were regularly recorded, shows a similar increase in the rate from 25 to 31; of course this may have been due to the patches of streptococcal pneumonia.

It is noteworthy also that Case VI complained of palpitation two days before death, and Case VIII evidently had an infarction three days before death. The occurrence of small infarcts in three cases in itself suggests primary thrombosis, for it is doubtful whether embolism could produce infarction in the few minutes which elapsed after the supposed impaction. The possibility of rapid and even instant death is admitted by those authors who believe in primary spontaneous thrombosis, still it is rather remarkable that death should occur so suddenly as obtained in our cases. But it must be remembered that thrombosis when once established probably progresses with an ever-accelerating rate, and, as Playfair points out, it is only when some sudden exertion was made, such as rising from bed or the like, calling for an increased supply of blood, which could not pass through the occluded arteries, that fatal symptoms manifested themselves.

Several of our patients died after exertion. Dr. Box's suggestion, that the acute symptoms may be due to the sudden displacement of the thrombi which have formed spontaneously in the pulmonary artery or right ventricle, is a reasonable one, though in Cases VI and VIII, the only ones in which loose thrombi were discovered, the patients lived about ten and fifteen minutes respectively after the first acute symptoms.

The evidence at our disposal indicates that thrombosis does not begin in the capillaries but in the smaller arteries, probably at their bifurcation (of the thrombi in the femoral veins in Case VII), and spreads in both directions but more slowly against the blood stream. The thrombi do not completely fill the larger vessels, and apparently only a portion of their circumference is actually in contact with the vessel wall. The coiling and twisting of the
thrombi upon which stress is laid is in itself no proof of embolism unless they can be unravelled, or unless sections prove that separate strands of an embolus have been glued together by encapsulating thrombosis. We believe therefore that thrombi may produce a coiled appearance in spontaneous thrombi as obtained in Cases i and vii.

Critics may suggest that the plugs in the pulmonary vessels are secondary to the impaction of an embolus. Apart from the fact that these hypothetical emboli must have been deposited in both lungs in seven cases, the time allowed for this secondary thrombosis is too short. Surely it is a question of hours, perhaps days—not minutes. No one believes that a stratified thrombi in the systemic veins—thrombus, for instance—progress at the rate of an inch or more in a few minutes. Why should they, then, in the lungs? The following are instructive cases of true embolism:

CASE A.—E.T., female, aged 21, with infective endocarditis of the pulmonary valves, was progressing favourably, when she died suddenly with slight cyanosis under five minutes. The brain was normal. An embolus derived from the pulmonary valve, 3 in. long, was found plugging the main branch of the pulmonary artery supplying the right lower lobe. Now, there was no thrombus in the neighbourhood of, or surrounding, this embolus; thus the blood had not clotted during the few minutes the patient survived its impaction.

CASE B.—C.R., female, aged 40, had double ovariectomy, June 10th, 1909, at the Liverpool Hospital for Women. Thirteen days later she awoke at 1 a.m., said good-night, and instantly died. A long ante-mortem thrombus, bifurcated at its extremity, which had undoubtedly come from the inferior vena cava and its branches, was found coiled up in the right auricle and orifice of the tricuspid valve; but it could easily be unravelled, as there was no encapsulating thrombus. The lungs were normal.

CONCLUSIONS.

In our experience spontaneous thrombosis is much commoner than embolism, in the proportion of 8 to 1 in some 1,000 post-mortem examinations from a single hospital. We believe an unaccountable acceleration of the pulse or respiration-rate, beginning in the second week after a major operation, especially abdominal, and associated with slight pyrexia, suggests the possibility of spontaneous pulmonary thrombosis and of a sudden fatal termination about the third week.

Cases of spontaneous thrombosis are overlooked from the assumption that only emboli can cause death in a few minutes, from neglect to remove and harden the heart and lungs together, and trace the distribution of the thrombi in situ in properly hardened specimens, and especially from neglect to make a thorough microscopical examination.

Even the association of pulmonary clots with others in the systemic veins does not necessarily prove that the former are embolic. Both may have arisen spontaneously and separately from similar causes.

This paper is necessarily incomplete, and many of the points raised are controversial; but perhaps it may cause surgeons who regard primary pulmonary thrombosis as a pathological curiosity to reconsider their position, and possibly to remodel their ideas.

BIBLIOGRAPHY.


DISCUSSION.

Dr. E. F. Baseford (London) drew attention to the light the intravenous injections of the emulsions of cancer throw upon the size of the thrombi which might be present ante mortem without evident signs of their existence. In these experiments it was necessary to inject an emulsion producing an immediate death that the pulmonary embolism. In the animals surviving the embolus grew, and in the course of time might be found as “thrombi,” blocking a large part of the pulmonary arterial system before invasion of the lung tissue took place. The same thing occurred naturally during the dissemination of cancer both in man and animals. Therefore it seemed to him that the extensive thrombosis described by Dr. Glynn and Dr. Box was not incompatible with its ante-mortem occurrence, provided it had developed gradually, whether it arose from embolus or spontaneously in the vessels.

Dr. C. R. Box (London) said it was gratifying to find that Dr. Glynn had been able to confirm the occurrence of primary pulmonary thrombosis as a cause of sudden death. In a paper read before the Clinical Society of London in 1905 the speaker had attempted to show that the suddenness of death in these cases was due to a process of embolism superadded to the pulmonary thrombosis, that portion of the clot which lay in the main pulmonary stem becoming detached from its anchorage in the heart and immediate branches of the pulmonary artery. That the coiled appearance of the clot in these cases was not always due to blood clots was shown by the fact that in one of the speaker’s cases the impacted clot bore the impress of the pulmonary semilunar valves.

A METHOD OF DISTINGUISHING DEAD FROM LIVE LEUCOCYTES.

By Professor C. ACHARD,

Paris.

I have undertaken, with two of my pupils, L. Raymond and Ch. Flandin, some researches on the distinction which may be drawn between dead and living leucocytes by using neutral red. Living leucocytes do not contain red-stained and intraprotoplasmic vacuoles or granulations. Dead leucocytes show their nuclei red-brown, and no intraprotoplasmic coloration.

The technique is very simple. Two solutions are used: the one is physiological salt solution with 5 per 1,000 of citrate of sodium; the other is a physiological salt solution with 1 per 1,000 of neutral red. We mix in a tube ten drops of each solution and add one drop of blood, or one to four drops of the sediment of a centrifuged exudate containing white cells; the tube is placed in the incubator and kept at the temperature of 37° C. for twenty minutes, then the liquid is examined in a glass cell, and the living and dead leucocytes are separately enumerated.

In the circulating blood there are no dead leucocytes, even in the most grave diseases. If occasionally a few dead leucocytes are seen, fresh preparations must be made again and examined within five minutes, in order to avoid accidental injuries of the white cells; then they generally yield no nuclear staining.

In various exudates dead leucocytes are not scarce, especially in cases of suppuration. In abscesses the number of dead leucocytes suddenly decreases after the incision. In acute meningitis, it appears that the reactions in the dead leucocytes are non-prognostic; their disappearance is a good sign, their increase an unfavourable indicator. Weill and Pollicard, of Lyons, who have recently controlled our method, have obtained similar results.

Of course, since the test of dead leucocytes is an anatomical method, it gives an account only of the local process—namely, of the local prognosis. But in acute meningitis the relationship of local to general prognosis is evident.

The red staining of the nucleus is also observed in recently dead and not yet disintegrated cells. Therefore a few stained nuclei are found in old suppurations, for instance, in tuberculous empyema, amongst a great deal of other indistinguishable remains.

In conclusion, we believe that this very simple method can be applied to pathological and clinical investigation. The remarks made applied to all leucocytes, more especially the polymorphonuclears.

THE EFFECTS OF ARTIFICIAL RESPIRATION ON THE STILLBORN.

By J. A. BRANTON HICKS, M.D., B.S., Lond., D.R.H.Camb., Assistant Pathologist to Westminster Hospital, S.W.

Putting aside the legal question, which is nevertheless an important one, as to whether the child was “born alive,” and admitting that the fetus has reached a stage of intra-uterine existence at which it might reasonably be supposed to live if born, there is no doubt that the proof of the act of respiration furnishes the best evidence of the child having lived at or about the time it was born.
The proofs that we have as to their disposal that respiration has occurred are derived (1) from a careful examination of the lungs, and (2) from an examination of the stomach and intestines. The lungs of a newly born child that has respired are found to present anteriorly on opening the chest, overlying the heart to a certain extent; they are bright pink and mottled, are crepitant to the touch, float entire, and when cut into small pieces, and attempts to completely empty the lung of air by pressure are useless. In the lung that has not respired the lungs do not present anteriorly in the chest on section, are thin and pink, brownish-purple, and sink in water. The stomach and intestines, or at any rate the stomach, of an infant that has breathed, contain air and float, whereas these organs in the fetus that has not respired sink, unless gases of decomposition may be present. This examination of the stomach and intestines is known as the second "life-test." Between these two conditions, which may be taken as typical extremes of inflation and non-inflation, there are any number of intermediate conditions of complete aeration, particularly in the first few days of life; and in addition there are two important exceptions to the statements I have made. The first exception is where the infant has respired but yet the lungs have all the characteristics of the fetal condition (complete atelectasis), and I remember an aneophaclitic monster that performed respiratory movements for some minutes, but yet the lungs were found in the fetal state post mortem. Cases also have been recorded in which this fetal condition of the lungs has been found in infants forty-five minutes and even longer after birth (Remer). A less marked condition of the fetal state persisting is where a portion or portions of the lungs are inflated, the rest being in the fetal condition (partial atelectasis), and slight degrees of this may be found in infants even of 5 or 6 months of age.

The converse condition is that in which the infant has never breathed, and yet appearances wholly or partly consistent with respiration have been found post mortem, produced by artificial respiration. It is to this case of that I wish to direct your attention, and I first propose to quote some of the cases investigated, and then see how far these findings coincide with the views of others. The first three cases to be now mentioned occurred consecutively:

**CASE I.**

A stillborn full-term fetus in which Silvester's method of artificial respiration and Schultze swinging were performed for one hour.

**CASE II.**

A stillborn fetus of a woman with a contracted pelvis, in whom labour had been induced at the thirty-fourth week. Schultze swinging and mouth-to-mouth respiration were performed for about forty-five minutes.

**CASE III.**

A stillborn fetus of the beginning of the ninth month. Mouth-to-mouth respiration was performed for half an hour.

In all these three cases the lungs were voluminous and presented anteriorly, they were pink and mottled, and the pleuræ were glossy. They floated entire and in pieces. The stomach and intestines contained air and floated.

It is interesting to compare these three cases with two others:

**CASE IV.**

A stillborn fetus, one of a pair from a case of Caesarian section in which artificial respiration could only be carried out for about ten minutes. Schultze swinging and mouth-to-mouth respiration were the methods employed.

**CASE V.**

The twin fetus to Case IV in which no artificial respiration was performed.

In Case IV the lungs were only partially inflated, but the stomach contained air. In Case V the stomach and lungs contained no air, and sank in water.

I will now show you the actual specimens from some of these cases, but the first remark that must be made is that the colours you now see before you do not represent their appearance in the recent state, for the pink and mottled appearance of the inflated lung has to a great extent disappeared in the process of preserving and mounting them, while the dull, non-inflated lung has become lighter on exposure to air, so that all three specimens of the lungs have somewhat the same colour.

The first specimen is a left lung from one of the first three cases mentioned (the other five lungs were cut up for the purposes of investigation), the second specimen is the partially-inflated lung of the fourth case, and the third specimen the non-inflated lungs of the fifth case. The differences in volume and condition of the lungs, when the upper lobes are compared, the three specimens showing a gradation from the fully-inflated down to the non-inflated condition. Under the microscope may be seen a piece of lung which was taken from the posterior portion of the lung, which, though not inflated, was pink and the air vesicles will be seen to be distended. The last specimen is a stomach from one of these cases containing air.

Considering now these appearances in their relation to the views of other investigators on this subject, there seems to be no disagreement as to the complete absence of the chest movements in the fetus that has not respired, and as to whether by artificial respiration the respiration of the lungs can be restored. The condition is that those of infant that has fully respired. Thus Casper denies that the lungs become pink and mottled, and as an example of this type of case I mention a case of my grandfather's (Braxton Hicks) in which the lungs answered all the hydrostatic tests, but were pale fawn in colour and not mottled. Taylor, in a series of experiments, came to the conclusion that considerable force must be used in introducing the air. Further it has been maintained that the air in artificially-inflated lungs only reaches the bronchi and bronchioles, and that persistent pressure will squeeze out the air and the lung will now float in water. In my cases the lungs were certainly pink and mottled, the air was not introduced by any greater force than is employed in the orthodox midwifery methods for artificial respiration, and air was also seen on the air vessels on pressure. Thus these findings agree most closely with those of Runge and Obolonsky, who found that, by the method of Schultze swinging, mottling and inflation of the air vesicles occurred, while by the method of the present case it is a total inflation. Further, it would seem that a simple method such as mouth-to-mouth respiration is capable of fully inflating the lungs (Case III), for Schultze swinging has been the one on which many investigators based their considerations.

Since, then, I hold the opinion that the appearances of complete respiration may be produced by artificial means, such a statement might be put forward as a defence in a case of infanticide, and I therefore propose to discuss how far such a defence could be made into a good one.

The points to be considered are:

1. Was it possible for the parturient woman alone to have carried out artificial respiration, or was there any one else there with her capable of doing it?
2. The method of artificial respiration employed.
3. The length of time the efforts at resuscitation were carried out.

As regards the time necessary to fully inflate a lung artificially I would submit that, by a comparison of Cases I and IV, twenty minutes is the least, but for obvious reasons it is not possible to experiment with regard to a time limit in cases of stillbirth. It seems incredible also that a jury could be persuaded that the parturient woman had herself performed artificial respiration on her child at birth, even supposing such a simple method as mouth-to-mouth respiration was the means suggested as being employed, and even supposing she was a woman who had some knowledge of midwifery, namely, a midwife or nurse. There is another type of case that might possibly give rise to difficulty, the following being an example that I have met with:

A woman in poor circumstances with a large family, and therefore perhaps not likely to welcome an addition to it, was delivered of a stillborn child while alone of what she stated to be a true date. The midwife, who had only been sent for a few minutes after birth took place, arrived in about ten minutes and proceeded, in the absence of the patient and resuscitation, to do artificial respiration and to note the appearances in the lungs and stomach of respiration having occurred were present post mortem.

Now though there was no particular reason in this case to suspect infanticide or that the midwife was an accomplice, yet since there are now a large number of women trained as midwives and therefore able to state that they are capable of performing artificial respiration, there is,
in my opinion, a possibility of an unscrupulous midwife being able to be a party to infanticide and successfully to assist in deferring the appearances found post mortem of complete respiration having occurred by a statement that she had performed artificial respiration for some time. The finding of change in the skin and umbilical cord or milk in the stomach, would of course negative her statement; but if the time when infanticide was committed was a short time after birth it might be difficult to prove the guilt of these people, and it was the case I have just mentioned that caused me to pursue the investigations I have laid before you. The conclusions I have therefore arrived at are as follows:

1. That lungs artificially inflated can resemble in every particular the lungs of complete respiration.
2. That the same statements hold good for the stomach and intestines.
3. That no excessive force in inflation is required to produce these effects, the ordinary midwifery methods being sufficient.
4. That a simple method, such as month-to-mouth inflation, will produce these appearances.
5. That for a lung artificially inflated to resemble a lung inflated by respiratory efforts, attempts at artificial respiration must have been carried out for at least twenty minutes.
6. That though as a defence in infanticide it may be urged that the appearances of normal respiration are due to artificial inflation, it would, however, be necessary to prove that the party charged were capable of performing the act of artificial inflation; (b) that the time was sufficiently long for inflation to have occurred; and (c) lastly, that no other signs of live birth were present.
7. That if all the conditions laid down are fulfilled (an event which I consider unlikely in a case of infanticide), there is no means of saying whether a child has normally expired or whether the appearances are due to artificial inflation.

In conclusion, I must express my indebtedness to Dr. Hobbs and Dr. Bernstein for the post-mortem notes on some of the cases mentioned in this paper.

THE VARIATION IN THE SIZES OF RED BLOOD CELLS.*

By Cecil Price-Jones, M.B. Lond.,
Pathological Department, Guy’s Hospital.

The variations in diameter of the red blood cells are conveniently observed and measured with the help of a camera obscura, the image of the microscope field being projected on to a table, when the individual cells can be outlined in pencil, and if the apparatus is adjusted for a magnification of 1,000 diameters, the cells may be measured with a millimetre scale, and expressed in terms of fractions of μ. Observations are made on blood films stained by eosin methylene blue ( Jenner’s stain). The sizes of cells vary from 4 μ to 12 μ, and are classified in groups progressing from 4 μ by 0.25 μ up to 12 μ. It is found that in a series of successive 100 cells the distribution of these groups is not constant, but if the average diameter is calculated at each successive 100, the figures are very approximate (Table I).

<table>
<thead>
<tr>
<th>Normal</th>
<th>Pernicious Anaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>μ</td>
<td></td>
</tr>
<tr>
<td>7.425</td>
<td>8.150</td>
</tr>
<tr>
<td>7.475</td>
<td>8.355</td>
</tr>
<tr>
<td>7.390</td>
<td>8.127</td>
</tr>
<tr>
<td>7.355</td>
<td>8.175</td>
</tr>
<tr>
<td>7.390</td>
<td>8.212</td>
</tr>
</tbody>
</table>

I have employed this method of average measurements in making comparative estimations of blood cell diameters in different specimens of blood. In normal human blood the red cell diameter varies from 6 μ to 8.75 μ, and the average diameter of five successive 100 cells is 7.4 μ. In the blood of pernicious anaemia the diameter varied from 4 μ to 11.75 μ, and the average diameter of five successive 100 cells was 8 μ. In normal rabbit blood the red cell diameter varies from 5.0 μ to 8.0 μ, and the average diameter is 6.4 μ.

When a rabbit receives under the skin 10 c.c.m. of a 1 per cent. solution of phenylhydrazine, the blood within twenty-four hours becomes brown and turbid, so that it is not possible to estimate correctly the haemoglobin per cent. and colour index. After six or seven days the brownness has disappeared and the blood is clear. It is then found to be very anaemic, 25 to 30 per cent. haemoglobin, and there is a markedly raised colour index. Similarly raised colour index is also observed in rabbit’s blood after severe haemorrhage though to a less degree, and after slight haemorrhage it is not observed, or is negligible (Table II).

<table>
<thead>
<tr>
<th>Colour Index</th>
<th>Phenylhydrazine</th>
<th>Bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>10 10 10</td>
<td>1.05 0.95 1.0</td>
</tr>
<tr>
<td>1 day after</td>
<td>10 10 10</td>
<td>1.2</td>
</tr>
<tr>
<td>3 days after</td>
<td>10 10 10</td>
<td>1.2</td>
</tr>
<tr>
<td>4 days after</td>
<td>10 10 10</td>
<td>1.2</td>
</tr>
<tr>
<td>6 days after</td>
<td>16</td>
<td>1.2</td>
</tr>
<tr>
<td>7 days after</td>
<td>15 15 15</td>
<td>1.2</td>
</tr>
<tr>
<td>10 days after</td>
<td>16 16 16</td>
<td>1.2</td>
</tr>
<tr>
<td>13 days after</td>
<td>16 16 16</td>
<td>1.2</td>
</tr>
<tr>
<td>15 days after</td>
<td>16 16 16</td>
<td>1.2</td>
</tr>
<tr>
<td>30 days after</td>
<td>16 16 16</td>
<td>1.2</td>
</tr>
</tbody>
</table>

If measurements are made on successive days of the red blood cells of a rabbit that has been treated with phenylhydrazine, it is found that for the first two or three days the average diameter is diminished, but that on subsequent days it becomes very markedly greater (Table III).

<table>
<thead>
<tr>
<th>Days of the Month</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Average diameter</td>
<td>6.5 6.4 6.4 6.5 6.9 8.0 8.5 8.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Similar variations in diameter were observed in the blood of rabbits after a moderately severe haemorrhage, but to a much less degree (Table IV).

<table>
<thead>
<tr>
<th>Days of the Month</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
<th>μ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Average diameter</td>
<td>6.5 6.3 6.2 6.5 6.7 6.4 7.3 7.3 6.7 6.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A. bled 25 per cent., B. bled 20 per cent., C. bled 9 per cent. of total haemoglobin.

* Towards the expenses of this research a grant was received from the Science Committee of the British Medical Association.
It is also to be noted that this increase in diameter of the red cells is synchronous with the appearance of large numbers of polychromic newly-formed red cells in the blood films.

Examination of films of bone marrow taken from rabbits killed at various dates after receiving phenylhydrazine, and after being bled, shows that the association of a raised colour index with increased diameter of red cell is connected with the formation of large nucleated red cells in the marrow. These cells, known as "metrocytes" or "mother cells," are not found in the marrow of normal animals; they are large cells 15 μ to 20 μ in diameter, with round or irregular contours, often showing poikilocytic processes suggesting amoeboid movement; the cytoplasm appears homogeneous and stains deeply and basophile, or more usually is polychromatophilic and of a purple-grey colour. The nucleus is large and stained deeply basophile, and presents various states of activity and division to form "daughter cells" or "megablasts.

Table V shows the percentages of the various kinds of nucleated red cells in the marrow of rabbits at different dates after treatment by phenylhydrazine.

Table V.—Analysis of Varieties of Nucleated Red Cells in Rabbit Bone Marrow after receiving 10 c.c.m. 1 per cent Phenylhydrazine Subcutaneously. (Twelve Rabbits.)

<table>
<thead>
<tr>
<th>Number of Nucleated Red Cells</th>
<th>Normoblasts and Primitively-Erythroblasts</th>
<th>Megablasts</th>
<th>Metrocytes</th>
<th>Free Nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15</td>
<td>41</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>1 day</td>
<td>41</td>
<td>42</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>1 day</td>
<td>44</td>
<td>33</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>3 days</td>
<td>33</td>
<td>29</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>3 days</td>
<td>31</td>
<td>17</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>5 days</td>
<td>44</td>
<td>35</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>5 days</td>
<td>36</td>
<td>29</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>8 days</td>
<td>68</td>
<td>23</td>
<td>12</td>
<td>0.3</td>
</tr>
<tr>
<td>8 days</td>
<td>58</td>
<td>14</td>
<td>6</td>
<td>1.6</td>
</tr>
<tr>
<td>8 days</td>
<td>41</td>
<td>11</td>
<td>7</td>
<td>1.8</td>
</tr>
<tr>
<td>11 days</td>
<td>149</td>
<td>28</td>
<td>14</td>
<td>0.9</td>
</tr>
<tr>
<td>14 days</td>
<td>61</td>
<td>44</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>14 days</td>
<td>126</td>
<td>38</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

Incidentally it may be noted that these metrocytes are commonly found in the marrow of pernicious anaemia, where the colour index and red cell diameter are also raised.

These metrocytes divide to form megaloblasts, the fact that many of the free nuclei found in the marrow of these animals seem to be megaloblast nuclei, suggest that the large-sized red cells have arisen from the immediate derivatives of metrocytes.

POLYCHROMASIA, AND THE PATHOLOGY OF HAEMATOMATA.

By G. R. WARD, M.R.C.S.,

In one of the most recent textbooks on diseases of the blood, there may be found the following statement:

"If blood, the erythrocytes of which are free from polychromasia, be defibrinated and kept in a sterile tube for four or five days and then re-examined, polychromasia will be found in many cells.

I am not concerned to dispute this statement, but to criticise the experiment upon which it is based and the deduction drawn therefrom—namely, that polychromasia is a change degenerative in nature. Defibrination is so gross a tampering with a tissue of such peculiar delicacy that it is impossible to disregard its influence. It is, therefore, basing argument on false analogy to argue the degenerative nature of intra vitam polychromasia from a consideration of changes produced by so damaging a process. When we consider that, in addition to this, the blood was kept in vitro for some time, the invalidation of the deduction quoted is only too plain. But since there is no such thing as a perfectly isolated, absolutely inessential action, we may be sure that there is some deduction to be drawn from this experiment. What, then, does it prove? Only that, if red corpuscles are kept for some time under conditions likely to prove deleterious, many of them will show an alteration of chemical constitution which may be demonstrated as "polychromasia." We are, then, forced to observe that this change cannot depend only on the surroundings, for the same alteration may occur in some cases even when the blood is subjected to experiment, or, to put it another way, even in normal blood polychromasia may be demonstrated under suitable conditions. This would appear to be a legitimate deduction from the experiment cited, and it is to be hoped that the essential chemical difference so demonstrated is in the nature of a degeneration.

With such consideration as a stimulant I examined the blood in haematomata following accidental and surgical injuries. In these the blood had been indeed removed from the circulation, but not from the body. The "living test tube" experiment which one finds dealt with in textbooks of physiology offers a close analogy; and, as in this case, the blood was not found to be clotted. Corpuscles under these conditions are naturally provided with an opportunity of exercising their normal functions; changes from disuse atrophy might be expected to ensue and finally death. As the conditions were the same for all corpuscles one looked for changes affecting all; wondering, at the same time, whether polychromasia would be seen in them, for only universal polychromasia would afford evidence of its degenerative nature. But again, as in the experiment first alluded to, polychromasia, when present, affected only a few corpuscles. There were, nevertheless, various changes affecting the corpuscles.

We find, then, that neither under the conditions of the first experiment nor under the more natural ones of the second, does the death of a corpuscle per se lead to a polychromatophic reaction. Does this reaction then, depend on the age of the corpuscle? Apparently not, for it was observed that we ought to find it in fresh drawn normal blood. It might be argued that cells about to become polychromatophile are removed from the blood, but we find that this does not take place even when the destruction is as its greatest— as in pernicious anaemia.

If, on the other hand, polychromasia were due to the youth of the cell, we ought to find it in normal bone marrow, which is not the case. The fact that this change is found in the young of certain animals is easily explained by supposing a congenital variation as its origin. The full development of the adult haemopoietic organs is not reached at the moment of birth; what more natural than that we should find in the young signs of a slow and differing activity?

If, then, we adopt as a hypothesis that there is a certain difference in the composition of the red corpuscles, congenital in origin and capable of being demonstrated under certain conditions as polychromasia, we can explain facts which did not at first appear to harmonise with it. I call it a "certain difference" because I do not wish to be understood as representing it to be an abnormality. Polychromasia is potentially present in normal blood, and may be demonstrated if the blood be first treated in a peculiar way—for example, by defibrination in the first experiment and by deprivation of normal surroundings in the second. It is noteworthy that the more unnatural conditions always render it demonstrable, the more naturally the more slowly, and by the reverse process.

In pathological conditions we may suggest that the potentiality is increased as the erythroplastic activity reverses to a more primitive type (for example, in regeneration), or, in other cases, that conditions—for example, of toxæmia—act as did the defibrination by rendering it more easily demonstrable; and polychromasia should be interpreted accordingly.

To sum up, I suggest as a working hypothesis that even in normal blood the corpuscles, as they show differences in size, in haemoglobin content, in isohoridity, show another difference known as polychromasia; that this difference
may be aggravated by conditions inimical to normal haemoglobinization; and may be rendered more easily demonstrable when the corpuscles are subjected to unnatural conditions. I further suggest that normal adult haemoglobinization produces this difference in a minimal degree, so that the incidence of other factors is necessary to its demonstration; and that it is to be expected in the blood stream in demonstrable degree when the marrow is of primitive type, either by reversion or lack of development.

If this hypothesis be employed, considerations of the approximate percentage haemoglobinization of their blood, and in the number of red cells per cubic millimetre observed by Fauss might, at any rate in part, have been due to a dilution of the blood. Seven rabbits were treated by the repeated subcutaneous injection of soap solution. Daily observations were made of the percentage haemoglobinization of their blood, and stained films were also examined frequently. The animals were finally killed and their total haemoglobin determined by Welcker's method. The results are shown in Tables I and II.

THE ACTION OF OLEIC ACID AND ITS SOAPS ON THE BLOOD.

By A. E. Boycott.

(From the Pathological Department, Guy's Hospital, London.)

Beginning with the discovery of an ether-soluble haemolytic substance in Bothrioccephalus and its identification as a chloro-ester of oleic acid, some importance has been attached to the presence of such substances, either in the lumen or in the wall of the alimentary canal, as being the effective cause of both hemolytic and idiopathic pernicious anaemia. It is, however, a far cry to transfer the results obtained in vitro with substances prepared from intestinal contents to the explanation of the presence of anaemias in vivo. Soaps of oleic acid, for example, are haemolytic enough, but there are reasons for supposing that they are really foreign to the economy and do not occur in the body except as the result of direct experimental introduction. Their haemolytic action in vitro is conditioned by a variety of circumstances, which make it difficult to predict what their action in the body would be; in particular, their concentration and the simultaneous presence of various other substances have to be taken into consideration. It seems likely, indeed, that a good deal of soap might be present in the circulating blood without causing haemolysis. It is certainly not difficult to show that the haemolytic effect of sodium oleate on fresh defibrinated rabbit's blood in vitro may be very much greater than that produced by the injection of corresponding amounts into the jugular vein of the anaesthetized animal. As regards the production of anaemia it seems necessary that the occurrence of blood destruction should be established, as well as the presence of haemolytic substances in the neighbour blood. While it is known that idiopathic pernicious anaemia is associated with excessive haemolysis, it is not clear that this obtains in Bothrioccephalus anaemia, and it is known that abnormal blood destruction does not occur in anaemias from other causes.

The question seems, therefore, to be primarily one of direct experiment, and Fauss has described how, by feeding two dogs with oleic acid and repeatedly injecting two rabbits with sodium oleate, he produced a marked degree of anaemia, and ultimately death. The immediate object of the present experiments has been to repeat these observations, with the addition that the total amount of haemoglobin has been determined, as well as its concentration in the circulating blood. It is obvious that the fall in the percentage of haemoglobin and in the number of red cells per cubic millimetre observed by Fauss might, at any rate in part, have been due to a dilution of the blood.

| Table I |
|-------------------|------------------|------------------|------------------|
| No. of Rabbit and Sex | Average Body Weight in Grams | Average Haemoglobin per Cent. of the Human Scale | Treatment |
| Before | At End | Before Treatment | During Last Week | Before Death |
| 3. F | 2075 | 2140 | 90 | 64 | 5 doses of 1 c.cm. 5 per cent. sodium oleate in 10 days = 5 grams |
| 4. F | 2090 | 1890 | 79 | 46 | 5 doses of 2 c.cm. 5 per cent. sodium oleate in 10 days = 5 grams |
| 5. F | 2785 | 2540 | 75 | 66.5 | 14 doses of 1 c.cm. 10 per cent. sodium oleate in 30 days = 14 grams |
| 6. F | 2655 | 2595 | 74 | 62 | 13 doses of 1 c.cm. 10 per cent. sodium oleate in 30 days = 13 grams |
| 9. F | 2595 | 2310 | 70.5 | 56 | 13 doses of 1 c.cm. 10 per cent. sodium oleate in 30 days = 13 grams |
| 11. F | 2545 | 2555 | 81.5 | 64 | 15 doses of 5 c.cm. 10 per cent. sodium oleate in 30 days = 6.5 grams |
| 12. F | 2615 | 2555 | 78.3 | 70.5 | 14 doses of 5 c.cm. 10 per cent. sodium oleate in 30 days = 7 grams |

| Table II |
|-------------------|------------------|------------------|------------------|
| No. of Rabbit | Total Haemoglobin in c.cm. Oxygen Capacity | Blood Volume, c.cm. | Per 100 Grams Initial Weight | Per 100 Grams Final Weight |
| 3 | 14.4 | 114 | 0.70 | 5.5 | 0.68 | 5.3 |
| 4 | 9.55 | 115 | 0.46 | 5.5 | 0.505 | 6.1 |
| 5 | 15.6 | 133 | 0.56 | 4.8 | 0.59 | 5.0 |
| 6 | 14.6 | 123 | 0.50 | 4.2 | 0.54 | 4.6 |
| 9 | 11.0 | 114 | 0.42 | 4.4 | 0.475 | 4.9 |
| 11 | 14.6 | 117 | 0.57 | 4.6 | 0.57 | 4.6 |
| 12 | 16.0 | 116 | 0.61 | 4.5 | 0.60 | 4.4 |

For comparison with these results we give also the figures for the total haemoglobin and blood volume obtained by the same method in a series of ten normal rabbits of about the same size (see Table III).

In all seven animals there was a fall in the percentage of haemoglobin, though in two instances (5, 12) this was not very great, and in only one case (4) was the figure reached lower than may be found in normal rabbits. The blood volume was normal, or somewhat increased. The total quantity of haemoglobin in the body was definitely less than normal in at least three animals (4, 5, and 9), and on the whole was deficient by about one-fifth. This result might be due either to increased destruction, deficient formation, or a combination of both factors. The

* The expenses of this work have been defrayed by grants from the British Medical Association and from the Royal Society.

† Made by neutralizing the impure oleic acid known as "Kahlbaum II."
signs of excessive blood destruction in the urine and organs (liver, spleen, kidneys, lymphatic glands) of rabbits have been elsewhere. The sum of these observations was to the effect that when a rabbit makes away with a large quantity of rabbit's red cells, the processes involved are an exaggeration of those normally at work. It was also pointed out that blood destruction is naturally so active in the rabbit that a moderate increase is difficult to diagnose from the examination of the urine during life, or of the organs after death. In the present series of animals, nothing could be found which definitely indicated any excess of blood destruction, but, owing to the circumstances which have been mentioned, this cannot be taken as showing that an amount of blood equivalent to the deficiency shown in the Welcker figures had not been destroyed. There is, however, clear evidence that the somewhat anaemic state to which these rabbits were reduced was not entirely due to blood destruction. No stage in the experiments did their blood show any histological signs of active regeneration of red cells. To any one familiar with the appearances, these signs are easy to detect after removal of quite small amounts of blood by bleeding, and especially if the blood is destroyed inside the body by such agents as saponin, distilled water, aniline, phenylhydrazine, and the like. Regeneration was therefore defective; whether destruction was going on at an abnormally fast rate is uncertain.

The post-mortem findings at the seat of inoculation were somewhat remarkable. Considerable solid masses of soap still remained unabsorbed, mixed with a clear or somewhat turbid liquid; histologically, there were great quantities of leucocytes, with some necrosis of the subcutaneous tissues. In most cases no definite abscess was present. In two animals, however (4 and 9), the subcutaneous mass was observed to increase in size rapidly for three or four days before death, and on post-mortem examination frank suppuration was found. One is inclined, therefore, to think that the soap injected was absorbed only in small part or not at all, and that the effects observed in the blood were due to inhibition of regeneration by the influence on the general health of the animal of the necrotic and inflammatory processes occurring locally rather than to any direct haemolytic effect of the inoculum. It is perhaps significant that the animals (4 and 9) in which the local condition was most exaggerated were those in which the deficiency of haemoglobin was found to be greatest.

Attempts were made to treat animals by repeated intravenous inoculations, but without success. Soap solutions are so irritating and cause such extensive

### Table III

<table>
<thead>
<tr>
<th>Sex</th>
<th>Weight in Grams</th>
<th>Haemoglobin per Cent.</th>
<th>Total Haemoglobin in c.c.m. Oxygen Capacity</th>
<th>Blood Volume, c.c.m.</th>
<th>Per 100 Grams Body Weight Oxygen Capacity</th>
<th>Blood Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.</td>
<td>1970</td>
<td>91</td>
<td>14.6</td>
<td>87</td>
<td>0.74</td>
<td>4.4</td>
</tr>
<tr>
<td>M.</td>
<td>2170</td>
<td>94</td>
<td>19.5</td>
<td>113</td>
<td>0.90</td>
<td>5.2</td>
</tr>
<tr>
<td>M.</td>
<td>2550</td>
<td>75</td>
<td>15.8</td>
<td>113</td>
<td>0.62</td>
<td>4.45</td>
</tr>
<tr>
<td>M.</td>
<td>2350</td>
<td>85</td>
<td>16.1</td>
<td>103</td>
<td>0.685</td>
<td>4.4</td>
</tr>
<tr>
<td>F.</td>
<td>2050</td>
<td>71</td>
<td>12.7</td>
<td>90</td>
<td>0.62</td>
<td>4.4</td>
</tr>
<tr>
<td>F.</td>
<td>2140</td>
<td>90.5</td>
<td>17.5</td>
<td>105</td>
<td>0.82</td>
<td>4.9</td>
</tr>
<tr>
<td>F.</td>
<td>2400</td>
<td>68</td>
<td>14.8</td>
<td>117</td>
<td>0.615</td>
<td>4.9</td>
</tr>
<tr>
<td>F.</td>
<td>2220</td>
<td>73</td>
<td>15.1</td>
<td>115</td>
<td>0.68</td>
<td>5.0</td>
</tr>
<tr>
<td>F.</td>
<td>2400</td>
<td>83</td>
<td>14.6</td>
<td>96</td>
<td>0.61</td>
<td>4.0</td>
</tr>
<tr>
<td>F.</td>
<td>2350</td>
<td>77</td>
<td>13.2</td>
<td>92</td>
<td>0.56</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.685</td>
<td>4.6</td>
</tr>
</tbody>
</table>

### Table V

<table>
<thead>
<tr>
<th>No.</th>
<th>Duration of Feeding</th>
<th>Sex</th>
<th>Weight in Grams</th>
<th>Total Haemoglobin in c.c.m. Oxygen Capacity</th>
<th>Blood Volume</th>
<th>Haemoglobin per Cent. of Human Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 days</td>
<td>M.</td>
<td>111</td>
<td>1.00</td>
<td>0.90</td>
<td>6.35</td>
</tr>
<tr>
<td>2</td>
<td>2 days</td>
<td>F.</td>
<td>117</td>
<td>1.27</td>
<td>0.90</td>
<td>6.50</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>F.</td>
<td>81</td>
<td>0.71</td>
<td>0.845</td>
<td>4.9</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>F.</td>
<td>62</td>
<td>0.70</td>
<td>1.04</td>
<td>4.1</td>
</tr>
<tr>
<td>5</td>
<td>48 days</td>
<td>F.</td>
<td>79</td>
<td>0.73</td>
<td>0.95</td>
<td>4.5</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>M.</td>
<td>103</td>
<td>1.125</td>
<td>1.05</td>
<td>6.7</td>
</tr>
<tr>
<td>7</td>
<td>50 days</td>
<td>F.</td>
<td>119</td>
<td>1.21</td>
<td>1.01</td>
<td>6.9</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>M.</td>
<td>85</td>
<td>1.11</td>
<td>1.31</td>
<td>7.35</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>M.</td>
<td>97</td>
<td>1.07</td>
<td>1.10</td>
<td>5.7</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>M.</td>
<td>85</td>
<td>0.90</td>
<td>1.06</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.625</td>
</tr>
</tbody>
</table>

### Table VI

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Weight in Grams</th>
<th>Total Haemoglobin in c.c.m. Oxygen Capacity</th>
<th>Blood Volume</th>
<th>Haemoglobin per Cent. of Human Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F.</td>
<td>116</td>
<td>1.22</td>
<td>1.05</td>
<td>7.1</td>
</tr>
<tr>
<td>2</td>
<td>M.</td>
<td>83</td>
<td>0.72</td>
<td>0.87</td>
<td>5.35</td>
</tr>
<tr>
<td>3</td>
<td>M.</td>
<td>110</td>
<td>0.92</td>
<td>0.84</td>
<td>6.7</td>
</tr>
<tr>
<td>4</td>
<td>F.</td>
<td>83</td>
<td>0.86</td>
<td>1.03</td>
<td>5.4</td>
</tr>
<tr>
<td>5</td>
<td>F.</td>
<td>100</td>
<td>0.93</td>
<td>0.93</td>
<td>6.2</td>
</tr>
<tr>
<td>6</td>
<td>F.</td>
<td>83</td>
<td>0.87</td>
<td>1.04</td>
<td>5.2</td>
</tr>
<tr>
<td>7</td>
<td>F.</td>
<td>90</td>
<td>1.00</td>
<td>1.11</td>
<td>6.3</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>102</td>
<td>1.65</td>
<td>1.03</td>
<td>8.0</td>
</tr>
<tr>
<td>9</td>
<td>M.</td>
<td>81</td>
<td>0.90</td>
<td>0.99</td>
<td>5.4</td>
</tr>
<tr>
<td>10</td>
<td>F.</td>
<td>97</td>
<td>0.90</td>
<td>0.92</td>
<td>5.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.65</td>
</tr>
</tbody>
</table>

### Table IV

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total Haemoglobin in c.c.m. Oxygen Capacity</th>
<th>Blood Volume, c.c.m.</th>
<th>Body Weight in Grams</th>
<th>Haemoglobin per Cent.</th>
<th>Total Haemoglobin per Cent. of Weight in c.c.m. Oxygen Capacity</th>
<th>Blood Volume per Cent. of Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>(M.)</td>
<td>14.95</td>
<td>119</td>
<td>2110</td>
<td>2350</td>
<td>76</td>
</tr>
<tr>
<td>B.</td>
<td>(M.)</td>
<td>7.7</td>
<td>63</td>
<td>1950</td>
<td>1510</td>
<td>80</td>
</tr>
<tr>
<td>C.</td>
<td>(M.)</td>
<td>7.4</td>
<td>55</td>
<td>1330</td>
<td>1170</td>
<td>84</td>
</tr>
<tr>
<td>D.</td>
<td>(M.)</td>
<td>14.3</td>
<td>118</td>
<td>2270</td>
<td>2355</td>
<td>78</td>
</tr>
</tbody>
</table>

### Table VII

<table>
<thead>
<tr>
<th>No.</th>
<th>Duration of Feeding</th>
<th>Sex</th>
<th>Weight in Grams</th>
<th>Total Haemoglobin in c.c.m. Oxygen Capacity</th>
<th>Blood Volume</th>
<th>Haemoglobin per Cent. of Human Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 days</td>
<td>M.</td>
<td>111</td>
<td>1.00</td>
<td>0.90</td>
<td>6.35</td>
</tr>
<tr>
<td>2</td>
<td>2 days</td>
<td>F.</td>
<td>117</td>
<td>1.27</td>
<td>0.90</td>
<td>6.50</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>F.</td>
<td>81</td>
<td>0.71</td>
<td>0.845</td>
<td>4.9</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>F.</td>
<td>62</td>
<td>0.70</td>
<td>1.04</td>
<td>4.1</td>
</tr>
<tr>
<td>5</td>
<td>48 days</td>
<td>F.</td>
<td>79</td>
<td>0.73</td>
<td>0.95</td>
<td>4.5</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>M.</td>
<td>103</td>
<td>1.125</td>
<td>1.05</td>
<td>6.7</td>
</tr>
<tr>
<td>7</td>
<td>50 days</td>
<td>F.</td>
<td>119</td>
<td>1.21</td>
<td>1.01</td>
<td>6.9</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>M.</td>
<td>85</td>
<td>1.11</td>
<td>1.31</td>
<td>7.35</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>M.</td>
<td>97</td>
<td>1.07</td>
<td>1.10</td>
<td>5.7</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>M.</td>
<td>85</td>
<td>0.90</td>
<td>1.06</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.625</td>
</tr>
</tbody>
</table>
did not like it. Animals A and B were fed for sixty-five days. 4 and D. for forty-three days, about 2 lb. of oleic acid being given in all. The animals were finally treated by the Weizler method. The total haemoglobin and blood volume were calculated on their body weights before feeding and on their average weight during the last week of the experiment. It was shown that any marked deficiency; this animal lost a weight of a great deal (20 per cent.), and the whole effect may well have been due to general malnutrition owing to its food being unsatisfactory in quality. It was found post mortem, with evidence of excessive blood destruction or active regeneration was found in any of these four animals. (See Table IV.)

Similar determinations were made on a series of similar rats kept as as possible under identical conditions except that they were not fed with oleic acid. (See Table VI.)

There is no suggestion here that the rate fed with oleic acid have less hemoglobin than the controls. There were no signs of active regeneration in films taken when the animals were killed.

**Summary.**

1. Rabbits repeatedly inoculated with soaps of oleic acid subcutaneously become anemic. Blood regeneration is defective; there is no clear evidence that blood destruction is excessive.

2. Rats fed with oleic acid showed no changes in the blood.

**References.**


**The Pathogenesis of Hereditary Haemophilia.**


Twelve cases were investigated. In all of them the coagulation of the blood was delayed. In those in whom the symptoms were most marked the blood took an hour or more to coagulate; in one case in whom the tendency to prolonged haemorrhage was less marked the coagulation time was thirty-six minutes, and in the slight cases it was about a quarter of an hour. This delay in coagulation is the only pathological factor which is constantly present, and it is sufficient to explain all the symptoms. There are good grounds, therefore, for regarding it as the proximate cause.

For the purpose of investigating into the reason of this delayed coagulation, blood was drawn into oxalate solution from the veins of normal and haemophilic persons, and after the subsidence of the corpuscles the plasma was pipetted off. Fibrinogen solutions were prepared by diluting the plasma with water, neutralizing with CO₂, and dissolving the centrifuged deposit in normal saline. Thrombin was prepared by expressing the fluid from the clot when calcium was added to fibrinogen. Thrombokinase was extracted from human tissues. Prothrombin was prepared by adding a minimal amount of thrombin to a fibrinogen solution, and expressing the fluid from the clot.

The first step was to determine whether the delay occurred during the first or the second stage of coagulation—that is, in the formation of the thrombin or in the action of the thrombin on the fibrinogen. It was found that haemophilic thrombin was as active as normal thrombin and that haemophilic fibrinogen was as readily coagulated as normal fibrinogen, but that the rate of formation of thrombin in haemophilic fibrinogen solution was much slower than in normal fibrinogen solution. The delay was therefore in the formation of thrombin. This might have been due to the presence of a substance which had a deterring influence on the process, but no such body was found, and haemophilic plasma did not inhibit the coagulation of normal plasma.

The cause was therefore to be looked for in connexion with the calcium, the thrombokinase, or the prothrombin. Varying quantities of calcium were added to haemophilic plasma, but no amount reduced the coagulation time to normal. Calcium was, therefore, not the cause.

With varying amounts of thrombokinase the same result was obtained, except when very large quantities were added, when coagulation was almost instantaneous in both normal and haemophilic plasmas. This is, therefore, not the cause. Further, there was found to be as much thrombokinase in normal plasma as in haemophilic plasma, and as much could be extracted from haemophilic blood corpuscles as from normal corpuscles. Thrombokinase was, therefore, not the cause.

There was no quantitative deficiency as regards the prothrombin in haemophilic plasma, but a qualitative difference was present, which showed itself in the unduly long time required by the haemophilic prothrombin to change into thrombin in the presence of calcium and thrombokinase. That this fault in the prothrombin was the sole cause of the delay in coagulation was shown by the fact that very small quantities of normal prothrombin when added to haemophilic plasma reduced the coagulation time to the normal.

The cause of haemophilia, therefore, was an inherited peculiarity in the constitution of the prothrombin whereby its activation into thrombin is retarded.

**Discussion.**

Professor Sahl (Bern) said that, as all present knew, he had found that the diminution of the coagulation power was a constant characteristic of haemophilia, and this was due to lack of thrombokinase. Traces of normal blood, if added to haemophilic blood, restored the coagulation power forthwith. In a later paper (Arch. f. exper. Path., 1911) he had shown that the difference between normal and haemophilic blood resided in the blood corpuscles. Normal blood corpuscles, even after careful washing, had a strong coagulating effect on haemophilic blood. His final conclusion, therefore, was that haemophilia was a cellular anomaly both of the blood corpuscles and the endothelial cells of the vessels.

**On the Absence of Altman's Granules from Cells of Malignant New Growths.**

(Second Communication.)

By Henry Beckton, M.A., M.D.Cantab, B.Sc.Lond., Assistant to the Director of the Cancer Research Laboratories, the Middlesex Hospital.

I.—Introduction.

In the paper published last year in this Journal, attention was especially directed to the fourth of the following conclusions which had been recently arrived at:—

1. Most kinds of normal cells contain Altman's granules, squamous epithelium and unstripped muscle constituting the chief exceptions.

2. In inflammatory conditions these granules are present in the various cell species concerned.

3. In innocent new growths these granules are present.

4. In malignant new growths these granules tend to disappear, or are absent entirely, from the particular type of cell involved.

A brief account was given of the granules occurring in normal cells, of which a few types were figured; certain inflammatory conditions were mentioned as having been investigated with the result that granules were obtained to the present in all these cases; and a description of the granules as regards granules being given of new growths, based upon an examination of 52 cases, 12 of which were non-malignant. Illustrations were also given of primary and secondary carcinomas showing groups of granular cancer cells surrounded by a ring containing lymphocytes, plasma cells, and other cells.

The number of cases of new growth has now been brought up to 117, and the conclusions already arrived at have been amply verified; these are now extended by the examination of a histological section of malignant disease formulated at the end of the present article.

II.—Normal Tissues.

As indicated above, the chief exceptions to the general rule that normal cells contain Altman's granules are
squamous epithelium and unstriped muscle. The collecting tubules of the kidney are also almost or entirely free from granules, but the first two are of fundamental importance on account of the large number of new growths into whose composition they enter, since opinions as to malignancy of a given tumour cannot be based upon the non-appearance of granules which are not present in the cells in which the tumour originated. It may be noted that in the early mammalian embryo the cells representing all three cell-species just mentioned show granules, but that these disappear during late embryonic and early extranerine life. Though fibroblasts contain fairly numerous small granules, their product, white fibrous connective tissue, usually shows none. Granules may be still present, however, although minute and few in number, in the connective-tissue corpuscles even when fibrillation has advanced to a very marked extent. Fig. 1 illustrates a normal tissue—it is chosen especially as showing normal granule-containing columnar epithelium which on becoming cancerous loses its granules (cf. Fig. 2).

III.—Inflammatory Conditions.

The following cases have been added to those mentioned in last year's paper, and are tabulated for the sake of comparison with the cases of new growth. The proportion

![Fig. 1. Normal endometrium. Granules are present in the columnar epithelium of the tubules, as well as in the cells of the surrounding tissue. Contrast presence of granules in normal endometrial epithelial cells in this figure with absence of granules from cancerous endometrial epithelial cells shown in Fig. 2.](image1)

![Fig. 2. Columnar-cell carcinoma of body of uterus. Granules are absent from the cancer cells; the stroma is infiltrated with granule-containing lymphocytes and plasma cells. Three lymphocytes, mapped out by their granules, are seen invading the cancerous epithelium. Contrast with Fig. 1.](image2)

![Fig. 3. Mycosis fungoides (skin). Low power photomicrograph. Section shows small round cells; note similarity to Fig. 5. In this case, however, granules are present. (See Fig. 4.)](image3)

![Fig. 4. Small portion of section illustrated in Fig. 3, highly magnified. Note presence of granules as in lymphocytes. (Zeiss apochromatic objective 4 in, compensating ocular No. 4.)](image4)

![Fig. 5. Lymphosarcoma (rectum). Low power photomicrograph. Section shows small round cells; note similarity to Fig. 3. In this case, however, granules are absent. (See Fig. 6.)](image5)

![Fig. 6. Small portion of section illustrated in Fig. 5, highly magnified. Note absence of granules from sarcoma cells, and presence of twelve lymphocytes marked out by their granules. Compare with Fig. 2, and contrast with Fig. 4.](image6)
of (?) guama (?) sarcoma, and although the general opinion of many who have examined a section stained with haematoxylin and eosin is that it shows inflammatory changes only, yet others have pronounced in favour of sarcoma. The granule findings, however, as given above, contrast markedly with those obtaining in the cases of spindle-cell sarcoma given in the table of new growths below. In the latter the patient has numerous cutaneous nodules on trunk, head, and limbs, and the case has been diagnosed varicose as mycosis fungoides and sarcomatosis cutis. The distribution of the nodules, the duration of the disease (several years), and the amenability to treatment (rapid disappearance of any given nodule under x rays) indicate inflammatory rather than malignant disease. Examined histologically by ordinary staining, the tumour presents simply the appearances of an aggregation of lymphocytes or of a lymphosarcoma; but while an undoubted case of lymphosarcoma (see table below) shows absence of granules, in this case granules are present, as indicated above (Figs. 3-5).

IV.—THE NEW GROWTHS

The granule appearances of additional cases of new growth investigated during the past year is given in the following tables. The proportion of cells containing granules is indicated as in the scheme given above; the cells referred to here, however, are only the essential cells of the new growths named, no account being taken of the granule-containing cells found in the stroma. It may be mentioned here that the absence of granules from the cells of a malignant new growth is seldom absolute, but the number of cells containing granules is usually not more than about 1 per cent, and such cells as contain granules often show at the same time indications that these granules are degeneration products of the cell protoplasm. In every case in the following tables the diagnosis was made without reference to the granule appearances found:

1. Non-malignant New Growth.

<table>
<thead>
<tr>
<th>Situation</th>
<th>Nature</th>
<th>Granules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid</td>
<td>Adenoma</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>Breast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parotid</td>
<td>Non-malignant endothelioma</td>
<td>+ + + +</td>
</tr>
<tr>
<td>Uterus</td>
<td>Hydatidiform mole</td>
<td>+ + + +</td>
</tr>
<tr>
<td>Rectum</td>
<td>Polypus</td>
<td>+ + + +</td>
</tr>
<tr>
<td>Thigh</td>
<td>Lipoma</td>
<td>+ + + +</td>
</tr>
<tr>
<td>Epididymis</td>
<td>Fibroadenoma</td>
<td>+ + + +</td>
</tr>
<tr>
<td>Thyroid</td>
<td>Adenoma</td>
<td>+ + + +</td>
</tr>
<tr>
<td>Rectum</td>
<td>Polypus</td>
<td>+ + + +</td>
</tr>
</tbody>
</table>

2. Spheroidal-cell and Columnar-cell Carcinoma.

<table>
<thead>
<tr>
<th>Situation</th>
<th>Nature</th>
<th>Granules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>Spheroidal-cell carcinoma</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>Rectum</td>
<td>Columnar-cell carcinoma</td>
<td>0 0 0 0 0</td>
</tr>
</tbody>
</table>

3. Endothelioma.

<table>
<thead>
<tr>
<th>Situation</th>
<th>Nature</th>
<th>Granules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>Lymphatic perithelioma</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>Orbit</td>
<td>Endothelioma</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>Hand</td>
<td>Lymphatic perithelioma</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>Breast</td>
<td>Perithelioma</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>Cervix uteri</td>
<td>Lymphatic endothelioma</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>Breast</td>
<td>Perithelioma</td>
<td>0 0 0 0 0</td>
</tr>
</tbody>
</table>

4. Sarcoma.

<table>
<thead>
<tr>
<th>Situation</th>
<th>Nature</th>
<th>Granules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectum</td>
<td>Endothelioma</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>Tongue</td>
<td>Lymphatic endothelioma</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>Breast</td>
<td></td>
<td>0 0 0 0 0</td>
</tr>
</tbody>
</table>

* Granules present in giant cells.

The above tables show at a glance the granule findings in the chief groups of new growths, and shows well the contrast between malignant and non-malignant neoplasms. A detailed table of all the new growths examined during the past year is given in the Archives of the Middlesex Hospital, Ninth Cancer Report, 1910; it includes also groups which may here be briefly remarked upon as follows:

5. Squamous-cell Carcinoma (11 cases) and Rodent Cancer (2 cases).—These are purposely omitted from the above tables, since Allmann's granules are absent from squamous epithelium (except very occasionally and in small numbers in the basal layer), so that one cannot in
the case of new growths originating in this epithelium speak of disappearance of granules, or regard absence of granules as indicating malignancy.

6. Teratoma (3 cases).—Granules are present in these as in other sarcomas; thus, at a spot at which there is an abrupt transition from columnar columnar epithelium to stratified squamous epithelium, the change from numerous fine granules to absence of granules obtains as normally.

7. New Growth of Ovary (3 cases) and Thyroid (1 case) described by Mr. Altmann. Ordinary histological examination of the whole of the tissues examined show presence of granules, except in one instance (columnar cell carcinoma of ovary). In last year's paper there is already an indication that new growths of these organs are anomalous, those which are histologically and histologically not ovarian, so called "malignant epithelium" in a term which is loosely used.

Possibly the absence of granules in the exceptional case just mentioned indicates that it was a secondary and not a primary growth of the ovary.

4. Ortho of Doubtful Histology (5 cases).—In some of these presence, in others absence, is the marked feature. In one case the diagnosis actually made was "carcinoma of rectum on macroscopic grounds; no histological evidence"; subsequent examination for granules showed absence.

Among the spheroidal-cell carcinomata tabulated above, the growth in which a majority of the cells contained granules was removed on a diagnosis of " (?) secondary nodule" from the skin of the abdomen of a patient whose bronchial carcinoma (situated on the side opposite to which the tumour was situated) had been removed eleven years previously. The presence of granules here may perhaps be correlated with the inordinate length of time elapsing before the appearance of metastases.

Sarcoma of the intestine has generally been found that the normal looking epithelium in the neighbourhood of the new growth is free from granules. The reason of this—proximity of malignant new growth, pre-cancerous condition, or other cause—is not obvious; in one instance, however, there was an abrupt change in a section from granule-containing epithelium of normal appearance to cancerous epithelium devoid of granules.

The Sarcoma—In all the different types of sarcoma examined absence of granules is a very marked feature. In the case, however, of myeloid sarcoma of the fibula the giant cells are for the most part crowded with minute granules, and stand out in striking contrast to the surrounding granuloma cells. The presence of these granule-containing cells, although comparatively few in number, is suggestive in connexion with the low degree of malignancy of myeloid sarcoma. (It may be remarked here that the almost exact converse of this condition obtains in a case of lymphosarcoma. Here the section from granules, but from most of the somewhat sparsely distributed several-nucleated cells granules are absent.) It is especially important in connexion with the sarcoma to bear in mind, when examining a section for granules, that these are absent from unstriped muscle and from fully organized "white fibros" connective tissue. Fibroid tumours occurring in various situations, and in particular fibromyoma of the uterus, may be instanced in this connexion.

V.—Conclusion.

On reviewing the results of examination of the various tissues mentioned in the foregoing, it is seen that the conclusions arrived at a year ago are fully confirmed, numerous additional examples which the tissue and new growths of not only the same but also new varieties falling into line with those already described. These conclusions may now be carried a step further. Setting aside new growths originating in cells not containing Altmann's granules, the neoplasms from whose cells granules are practically absent is quite clear; these belong to the group of undoubtedly malignant new growths. The position of those neoplasms in which presence of granules is the conspicuous feature is not quite so plain; most of these examined are definitely non-malignant, while growths originating in the ovary and the thyroid body admittedly present anomalous features in respect of malignancy. On the whole it appears that the absence or presence of Altmann's granules may be used as a histological test ("granule test") for malignant disease and other conditions as follows:

1. Excluding new growths originating in cells not normally containing Altmann's granules, absence of granules in all or nearly all the essential cells of a new growth indicates malignancy.

2. Presence of Altmann's granules in all or nearly all the essential cells of a new growth is usually associated with non-malignancy or only with malignancy of a special kind or limited degree (cf. thyroid, ovary).

3. In a tumour the diagnosis of which lies between inflammation and sarcoma, presence of Altmann's granules indicates the former, absence the latter.

CHLOROFORM NECROSIS OF THE LIVER.

By G. W. Goodhart, M.A., M.B.,
Gull Student in Pathology at Guy's Hospital.

The experiments which are the subject of this communication were not originally made for the purpose of investigating the production of necrosis of the liver by chloroform. They were but a means to a quite a different end, but they seem of sufficient interest to record, fragmentary though they be, for they approach the subject from a somewhat different standpoint, that on which the whole process of necrosis should be studied. Whipple and others, further, although a good deal of work has been done on the subject, it would not seem to have attracted any very general attention.

It is well known that chloroform, if given to an animal in sufficient dose, may, and under certain conditions invariably does, produce a marked change in the liver. The change, originally described as a fatty degeneration, has been defined by later investigators as a necrosis of the cells at the centre of the lobules. Most of these previous experiments appear to have been conducted in such a way that what big doses of chloroform; in the very carefully planned series of experiments of Whipple and Sperry[1], most of the dogs used were anesthetized for periods of one and a half to three and a half hours, usually twiced and often three times on days in quick succession. Although these observers concluded that necrosis of the liver invariably resulted in dogs after an hour or more narcosis with chloroform, none of the experiments actually recorded deal with a single anaesthesia of less than two and three-quarter hours' duration.

It was my aim to see whether equally constant changes, in less degree perhaps, could be produced by smaller doses, and, if so, what proportion of cases recovered when any marked change had been produced. Whipple and Sperry, in their report that when recovery does take place repair of the liver is complete, even after a most extensive necrosis, in three weeks, and leaves behind no trace of the necrosis. In my experiments rabbits and rats were used, but the use of rats was discontinued owing to the frequent occurrence of focal necrosis in the liver of rats from apparently independent causes.

The experiments here recorded deal only with rabbits. Chloroform was given by inhalation and by subcutaneous and intraperitoneal injection. The inhalation experiments will be dealt with first.

The 10 animals used were anesthetized first with ether until they were well under, and chloroform was then continued. Only a very slight depression of anesthesia was maintained, the cornel reflex being present practically throughout the experiments. The administration was continued from periods varying from thirty minutes to two hours, and two of the animals were anesthetized in an unusual manner. One animal was operated on in the way the animals took the anesthetic. Nearly all took it badly, and in three cases, in spite of the presence of a brisk cornea reflex, the animal suddenly stopped breathing, and artificial breathing was necessitated. The results are tabulated in Table I.

One animal died during anesthesia after forty-five minutes' narcosis. No change could be found in the liver attributable to the chloroform. Some fine globules of fat

* The expenses of this research were in part defrayed by a grant from the British Medical Association.
might suggest that the effect on the liver varies directly with the dose; this is, however, not borne out on closer examination; for example, Rabbit 5, which underwent the longest period of anaesthesia, appears half-way down the list, and moreover in the case of Rabbits 1 and 4, which head the list, the mixture of air and chloroform circulating through the facepiece of the apparatus contained a much lower percentage of chloroform than was the case of Rabbits 2 or V.3.

This apparent lack of correspondence between dosage and the effect on the liver confirms the impression formed from observation during the anaesthesia, namely, that the toxic effects of the drug in no way run parallel to the anaesthetic effect, and I quite agree with those observers who say that the degree of anaesthesia is no measure of the degree of poisoning; twitching of the limbs, rotatory movements of the jaw, and very rapid breathing may all occur as signs of a deep degree of poisoning in only a very light stage of anaesthesia.

Injection Experiments (Table II).

Eight animals received 0.5 c.c.m. of chloroform subcutaneously; of these six died within three days, and all six showed an extreme form of necrosis at the centre of the lobules. It may be questionable as to whether it is permissible to say, from the histological appearances, that a particular cell is dead and beyond recovery—that is, that necrosis has taken place; but in these animals there can, I think, be no doubt whatever. The centre of the lobules (in many cases as much as three-quarters of the entire lobule, or even more) is represented by a mass of red blood cells, with the remains of isolated liver cells scattered about amongst them. Some of these liver cells are intact, with nucleus and surrounding protoplasm. Around the periphery of the lobule are two or three layers of healthy-looking cells. Droplets of fat are scattered throughout the lobule, but they are much more densely packed in the ring of cells lying immediately internal to the healthy peripheral cells. The cells of the lobule are dilated, but there are few leucocytes.

In Rabbit V.3, anaesthetized for seventy minutes, the necrosis is limited to a narrow ring of cells surrounding the central vein, but the cells external to this ring, forming more than the inner half of the lobule, are loaded with droplets of fat.

In Rabbit V.1, anaesthetized for forty minutes, there was no necrosis, but the cells of the inner half of the lobule were densely packed with fat, the remaining cells at the periphery being practically free.

In Rabbit V.2 the fatty change was much less marked, although strikingly similar in distribution.

In 77 per cent. of these animals which survived the period of anaesthesia there was therefore a marked change afflicting principally the cells at the centre of the lobule. A rough glance at the accompanying table (Table I).

<table>
<thead>
<tr>
<th>No. of Rabbit</th>
<th>Length of Anaesthesia</th>
<th>Time of Death</th>
<th>Condition of Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75 min.</td>
<td>KILLED IN 48 HOURS</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>DIED IN 48 HOURS</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>KILLED IN 48 HOURS</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>2 hours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>12 min.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V.1</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V.2</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V.3</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V.4</td>
<td>Died during anaesthesia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V.5</td>
<td>20</td>
<td>KILLED IN 48 HOURS</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Definite necrosis of centre of lobules.
= = No necrosis of centre of lobules.

The times are arranged in order according to the degree of change found in the liver.

TABLE II.—Eight Animals Injected Subcutaneously with 0.5 c.c.m. of Chloroform.

Died within 20 hours...2+ Fat in intermediate zone.
Died within 24-48 hours...2+ More fat than in preceding.
Died within 2-3 days...2+ Fat in greatest quantity.
Killed on sixth day...1+.
Killed on sixteenth day...1+.

+= Definite central necrosis.
= = No central necrosis.

Injection of 0.2 c.c.m. of Chloroform.—The chloroform was injected either subcutaneously or intraperitoneally. I have not been able to trace any difference in the results.
obtained from the two methods, and for convenience sake they are here grouped together (Table III).

The results of this series are much more complicated, and I shall only state them briefly. I cannot attempt to explain them at present. None of the animals of this series showed any anaesthetic effects from this dose. Within a few minutes of the injection they were usually found to be lying stretched out with the head retracted, and breathing shallowly and with great rapidity; this condition lasted not infrequently for several hours; but they responded to stimuli always, and if their position was altered, they would usually take a few jumps round the cage before returning to their original position.

Thirty-four animals were injected with the same dose twenty-six days later; of these, 1 died within twelve hours, 1 within ten days, and 2 within twenty-six days. None of them showed any central necrosis or fatty degeneration. The remaining 31 animals were again reinjected, the survivors receiving a fourth injection, and so on; only 1 animal survived a sixth injection, and this was killed. The results are seen in Table III.

Table III.—Thirty-four Animals Injected Subcutaneously and Intrapertioneally with 0.2 c.c.m. of Chloroform.

<table>
<thead>
<tr>
<th>No. of Injections given.</th>
<th>No. of Rabbits used.</th>
<th>Died within 12 Hours.</th>
<th>Died or Killed within 10 Days.</th>
<th>Died or Killed within 26 Days.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>0</td>
<td>2 died</td>
<td>5 killed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>1</td>
<td>1 died</td>
<td>2 died</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>0</td>
<td>4 died</td>
<td>2 died</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4*</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>0</td>
<td>1 killed</td>
<td>1 died</td>
</tr>
</tbody>
</table>

+ and — as before.  
* = fatty degeneration at centre of lobule.  
Two of these animals were not used further.

In none of these reinjected animals was any change found in the liver at all comparable to that found in those dying or killed after a first injection. These animals did, however, show a definitely increased mortality; other animals not injected and kept under the same conditions lived on happily, while the chloroform rabbits died off. The livers of these animals, although in no way resembling those of the animals of the previous series, do all present a more or less similar picture, and one that must, I think, be abnormal. First, they are usually very full of blood; the central veins are dilated and packed with red blood cells; radiating from these are greatly distended capillaries also full of red cells, and compressing the adjacent columns of liver cells, so that in many cases the field presents a picture of broad columns of blood cells separated by a mere chain of nuclei of liver cells connected only by an attenuated cord of protoplasm. Secondly, the central vein is often lined by a thick wall of connective tissue frequently of a thickness of three liver cells, whilst in other cases the outline of the central vein has entirely disappeared and is represented by an irregular space without a definite wall, filled with a grey granular material which often contains small colonies of isolated liver cells embedded in it. Here, too, the red blood cells form a very striking picture as those between the columns of liver cells, and often in close proximity to the central vein there are definite areas of haemorrhage.

Various observers, especially in France, speak of a second form of change in the liver produced by chloroform narcosis. This is represented by a whole mass of which may, perhaps, resemble the conditions here described. For a satisfactory explanation of this condition I am completely at a loss; but it is possible that there has originally been some destruction of the cells at the centre of the lobule, and that in the process of regeneration of the liver cells the re-formation of the central vein has been incomplete or in some way interfered with.

The method of repair, and the extent to which it occurs, is too lengthy a subject to be discussed here, and must be left for a future occasion.

COMBINED SECTIONS OF PATHOLOGY AND BACTERIOLOGY.

The Section of Pathology hold a joint sitting with the Section of Bacteriology for the purpose of considering complement-fixture in diagnosis. It resulted in the reading of the following papers on different aspects of the subject:

II.—Professor A. Wassermann, Berlin.

THE DIAGNOSTIC USE OF THE COMPLEMENT-FIXATION METHOD.

The theme before us has become so very voluminous during the past few years through the zealous work of different authors that I cannot hope to treat it exhaustively within the limits of a short discourse. I shall, therefore, only pick out what is most important practically, and present even that as briefly as possible, since I regard my paper merely as an introduction to the discussion to follow.

In the first place, regarding the nature of the so-called fixation of the complement, it is known to you that it depends upon this principle: that when an antigen is mixed with its homologous immune body a union occurs between the two. If now complement—a constituent of every fresh serum—be at the same time anchored through the union of the antigen and antibody. It follows accordingly that if the complement be anchored, the conclusion may be drawn that either the homologous antigen or the homologous immune body is present in such a mixture. The determination whether in such an experiment the complement is bound can be made easily and convincingly.

For this purpose one needs simply to add simultaneously or somewhat later the serum of an animal, which has been previously treated with red blood corpuscles, the so-called amboceptor, together with its homologous erythrocytes. If the complement has already become bound as a result of the union between the anti and immune bodies, then it is no longer available for the haemolytic amboceptor and the red blood corpuscles. Consequently the latter remain undissolved. But if, on the contrary, fixation of the complement has not occurred, the complement goes over to the second group, and causes haemolysis of the blood corpuscles. Thus from the appearance or non-appearance of haemolysis, one can draw the conclusion whether the sought-for antigen or immune body is present in the first group. This experimental method has been known for only about ten years, and was first announced by Bordet and Gengou in connexion with their tuberculin research work. These authors, however, had already recognised the diagnostic value of their method, for they employed as antigen emulsions of various kinds of bacteria with which they mixed human and animal serums, in order to determine whether fixation of the complement occurred—that is,
whether antinutritional substances corresponding to the different bacteria were present in the sera. Such investigations were made with typhoid, Bordet and Gengou with bovine and avian typhoids.

Nevertheless, the method as such could not establish itself in practical medicine, and for the reason that, as reported by Bordet, it offered no advantages over the diagnostic results already in vogue. As already mentioned, Bordet used bacterial emulsions as antigen. Consequently the method in this form could only be employed in those infectious diseases whose agents could be grown in pure cultures. A modification, however, was made in this respect by those of such patients practising Dr. Bruton; to this end he was able to demonstrate that a bacterial emulsion is not always necessary as antigen, but, on the contrary, it is even more advantageous to use as antigen the dissolved substances of bacteria which I obtained by the extraction of the bacilli bodies. One is much helped in the understanding of this through the excellent works of your late lamented countryman, Macfadyen, who demonstrated that the products of bacterial solution are often, biologically considered, even faster than the bacteria themselves. What, however, marks the greatest advance in my lab is that from that time on one might also use this fixation of the complement method in those diseases whose agents are still unknown or unable to be cultivated. To this end, in place of the bacteria, one only needs to make extracts of the pathological organs of the disease in question, and to use them as antigen.

After the preliminary investigations which I made on the extracts of meningococci and on tuberculosis, namely, the extract of tubercle bacilli, had demonstrated that for this purpose I could use an alcoholic extract from normal organisms, while, on the contrary, it reacted typically with the aqueous extract of syphilitic organisms. It follows, therefore, that in scientific and responsible researches one should always work with the original antigen obtained from syphilitic organs.

Before I pass to the application of serum diagnosis to syphilis, I should like to say a few more words about the further modifications of the original technique. It is an old story to you that in the past few years many other modificaions have been made toward the original serodiagnostic method of syphilis. One author has proposed the use of a different complement from the one I recommended; another author a different haemolysis system—so to say, a different method—whereas another has used horse blood corpuscles, with its homologous rabbit immune body which I employ. All these modifications aim at making the method more sensitive. We purposely chose a method that should not be too sensitive in order to be able to discover whether the reaction substances in the serum of the syphilitic, in order to avoid having a non-syphilitic give the reaction, and thus be regarded as a syphilitic. On the other hand, it is freely admitted that among 100 syphilitics an equal number might give the same reaction as a non-syphilitic one. Therefore, the method we used has been passed over by the reaction. We regard this, however, as the lesser evil, since such individuals can be re-examined after a time, and particularly since physicians are not accustomed to exclude the diagnosis because of a negative diagnostic reaction.

Now, most of the authors who proposed this and that modification of the original method, sought to make the system so sensitive that it should show a still higher percentage of positive reactions than the original method. With this aim one author proposed the use of normal heat serum, another, as Noguchi, for example, the use of human blood corpuscles instead of the heat serum; this method, however, is not recommended for practice. This method, particularly to Noguchi’s procedure, as Slesak, working in my laboratory, has just recently demonstrated through careful tests. It suffers just like the other methods from the fault that it is too delicate, in that it gives reactions with the serum of patients having other diseases. The original method shows no reaction in these cases. I cannot, therefore, recommend the use of these procedures or modifications for practice. In compliance with numerous requests in the literature and from practising physicians, I have seen to it that the reliable reagents, which according to my point of view are needed, should be dispensed from a central office, namely, the Pharmacological Institute of Ludwig Wilhelm Gans in Frankfurt-Maine.

Concerning the diagnostic significance of the syphilis reaction, this has been settled in the extraordinary way in every country that I do not need to speak much about it. In many countries, as in Austria and Denmark, serodiagnostic institutes have already been established by the Governments. I myself with my co-workers have carried out more than 10,000 reactions, and by our method we have never yet made a false diagnosis. To be sure, to accomplish this one must completely master the technical details of the reaction, must have faultlessly accurate reagent titrations, and must be able to carry out the reaction exactly. So-called partial arrests of cell degenerations can likewise occur in other diseases; or, on the contrary, through only slight extension of the syphilitic process—as, for example, a gumma of the bone—degeneration worthy of notice does not take place. It follows, therefore, unconditionally, that the extract of syphilitic organisms must be the more specific and reliable antigen. Specific evidence in this connexion has been come in the recent experiments made by my countryman, Dean—who is at present working in the Lister Institute here—experiments concerning the relationship of idiocy to congenital syphilis, which he carried out in my laboratory. Dean was able to show that the organism of syphilis is practically never obtained in the aqueous extract of normal organisms, while, on the contrary, it reacted typically with the aqueous extract of syphilitic organisms. It follows, therefore, that in scientific and responsible researches one should always work with the original antigen obtained from syphilitic organs.
Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.

Complement Fixation Method.
of blood can be settled as to their origin, whether human or animal.

Thus we see what a great new field this method of complement fixation has opened up to us, and how animal research and experimental laboratory work have again succeeded in giving importance to diagnosis and therapy to practical medicine and to the clinic.

II.—H. Wansley Batly, M.A.Camb., M.R.C.S., L.R.C.P.,
Bacteriological Department, St. George’s Hospital.

THE PRACTICAL VALUE OF THE WASSERMANN REACTION.

I venture to lay before you the result of eighteen months’ use of the Wassermann reaction at the London Lock hospitals. I have completed over 1,000 determinations, and as the months pass on I am only more and more impressed with the value of the test. I have used an alcoholic extract of rabbit's heart for syphilic antigen, and have found that it has contained as good results as with an alcoholic extract of fetal syphilitic liver.

Many observers have tried to simplify the test and obviate the necessity for using rabbits and guinea-pigs by making use of the complement and haemolysin for sheep's corpuscles in human blood serum. It seems to me, however, that the most essential of all factors in a comparative test such as this is that all factors except the one to be tested should be constant. With Hecht’s, Bauer’s, Stern’s, or Fleming’s technique, however, variants are included which, in my opinion, does not increase the reliability of the test; for the complement content of human serum varies greatly, and some human sera contain no haemolytic antibody to sheep's corpuscles, while others contain some, but in varying quantities. I use and advocate the technique that I use and advocate, that I mix fresh guinea-pig serum (for complement) and alcoholic extract of rabbit heart (for the body acting as syphilitic antigen) and normal saline solution in bulk and put 2.5 c.c.m. of this mixture into each test tube. The only variation I use is the decomplemented serum to be tested, of which I add 0.3 c.c.m. per tube.

In the same way I mix in bulk the prepared rabbit’s decomplemented haemolytic serum and the suspension of antigen that I have obtained as good results. Any difference in the amount of haemolysin between the tubes must therefore be due to the only variant factor—namely, the serum tested, for syphilitic antigen complement and haemolytic system are all constants. This is not the case, however, with the simplified method, where the complement and haemolytic antibody are variants, and the cause of any difference in haemolysis must therefore be unknown.

Benefiting by the advice and experience of Captain Harrow, I also published a very interesting article on Serum Diagnosis of Syphilis in the July number of the Journal of the Royal Army Medical Corps, I have for the last six months used two strengths of complement, and so obtained a roughly quantitative measurement of the complement deviating power of the serum. Frequently in this way we can obtain an indication that the patient is getting under the influence of treatment, and some haemolysis will occur in the tube containing the larger dose of complement, although the smaller dose of complement is completely deviated.

By this means I have investigated the therapeutic value of some of the different antisyphilitic remedies, and the comparative value of the different methods of administering mercury, and in conjunction with Mr. Charles Gibb I published in the Lancet of May 7th a short preliminary note on this subject.

The results that I have so far obtained point to the conclusion that less than six months’ pill treatment has little or no effect on the Wassermann reaction, no trace of haemolysin even in the tubes containing the double dose of complement. On the other hand, a large majority of cases treated by inunction and a very considerable percentage of cases treated with intramuscular injection of insoluble mercurial preparations show some definite effect of treatment after three months’ course.

*This quantitative measurement of the complement deviating power of the serum can obviously not be determined by the so-called simplified methods.

Potassium iodide and the arylarsanates seem to have no effect on the Wassermann reaction as far as three months’ treatment is concerned.

We therefore conclude that inunction and intramuscular injections are the best methods of administering mercury and therapy to patients with the order named. I have found that about 85 per cent. of all untreated syphilis give a positive reaction. In no case of tuberculosis or cancer and in no healthy individual have I obtained a positive reaction. The highest percentage of positive results has been obtained with untreated congenital syphilis, practically every case giving the reaction.

In primary cases in which the lesion has been present for less than a fortnight the majority of cases give a negative reaction, but after the lesion has been present for a month a positive reaction is recorded in 75 per cent. of cases.

Secondary syphilis with symptoms gives a positive reaction in over 50 per cent. of cases and tertiary syphilis in about 75 per cent. of cases. Of parasyphilitic conditions general paralysis gives a positive result in about 50 per cent., but only in about 50 per cent. of cases.

The history of the Wassermann reaction is too recent for us yet to have acquired sufficient data in regard to its value as a guide to permanent and complete cure. Even in a case where a succession of negative results has been obtained after all treatment has been left off, we cannot be certain until another twenty years have passed that the disease is entirely and absolutely obliterated and that no later manifestation will ever occur. By its means we have indeed learnt that many cases are considered cured and it is the general opinion that three negative reactions taken at three or six months intervals, during which no mercury is given, can be considered as an absolute cure.

I can only say that an opinion based on this evidence is of far greater value than an opinion based only on clinical or rule of thumb grounds, but we cannot yet be certain that such an opinion would invariably be correct.

In the state of our present knowledge, however, I do not think that it would be justifiable to recommend that the Wassermann reaction be used in the diagnosis of syphilis for at least a year after the patient has been given a three years’ treatment, even if a succession of negative reactions have been obtained, for it has been shown that if treatment is left off a positive reaction may occur. Too much reliance, therefore, should not be placed on a solitary negative reaction for the conclusion of syphilis.

We at the Lock hospitals are entirely satisfied as to the great value of the Wassermann reaction from the diagnostic point of view; such clinically doubtful cases that gave a negative reaction always showed later either by their reaction to mercurial treatment or by the development of clinical symptoms that they were syphilitic. In so far as we have gone at present in regard to treatment also the pathological evidence as estimated by the Wassermann reaction and the clinical evidence seem to be in accord; the majority of cases to go hand in hand. I consider that more reliable results are obtained with blood serum than with cerebro-spinal fluid. I have also failed to obtain satisfactory results when urine has been substituted for serum.

As regards the complement-deviation test for tuberculous infection as used by Dr. Marmorek, of Paris, my results have been disappointing, and have not agreed with the results published in Paris. But with the Wassermann reaction it will be seen that my results are in accord with those obtained by Professor Wassermann.

III.—Robert Muir, M.D.Edin.,
Professor of Pathology, University of Glasgow.

THE FIXATION OF COMPLEMENT IN GENERAL.

As a contribution to this discussion, I propose to offer some observations with regard to the properties of complement in general, especially in relation to the process of fixation. Complement is notoriously susceptible to physical agencies, heat, light, and even mechanical shaking, and to the action of chemical substances; it is no less strikingly liable to absorption by various substances in solution or in suspension, and it is accordingly desirable to consider some of the chief conditions under which this takes place.

It may, however, be well in the first place to point out that in the various tests we rarely inquire into the presence or absence of complement in an active form.
Now, when complement or its activity disappears from a fluid in which it was formerly present, this may be due to one or at least three conditions: (a) Destruction of complement—the natural physical or chemical decomposition of complement—for example, by a concentrated salt solution, the activity being restored on diluting, and it is important to bear in mind that many proteins in solution have a certain amount of their activity fixed and that by such a method, when suitable controls are used, the absorption or fixation of a very minute quantity of complement can be readily observed.

(b) Inhibition of the deviation of complement when one of the two substances is present in excess. An example of such an occurrence is seen in the case of a precipitating antiserum produced by the injection of the serum of one animal into another of different species. In this case an excess of antigen leads to a diminution or even disappearance of the deviation of complement, a phenomenon which is closely allied to the inhibition of precipitation and to the solution of precipitate after it is formed.

(c) Inhibition of the deviation of complement in the case of all three of the chief types of antiserums—namely, antibacterial, haemolytic, and precipitating sera—and I can testify to its extreme usefulness in the study of organic substances, bacteria, red corpuscles, tissue cells, and so on. In the process of studying variations in their amount under altered physical conditions, by this method I was able to study the actual amount of complement taken up in relation to the amount of immune body, and was able to show that many times the haemolytic dose might actually be fixed. Again, Dr. Browning and I applied the method to the study of the combination of various complements, and showed how different substances combined with different animals showed great variations in their toxic effects, as seen in haemolytic phenomena. We also showed how complements from different species of animals mutually excluded one another from combination with sensitized corpuscles, that they inactivated one another in small groups—and such observations have since been widely extended. In the case of a precipitating antiserum, Dr. Martin and I confirmed the results of others as to the deviation test being much more delicate, often ten times more so, than the precipitin test, and Dr. Haber and Wilson applying the method to albuminous urine, found that albumen could be demonstrated by it in from ten to a hundred times greater dilutions than was possible by any chemical method. The method can be applied, with little or no alteration, to the study of the physical properties of the serum. In this case the bacteria sensitive to bactericidal action are substituted for sensitized corpuscles. Accordingly, as a means of analysis of haemolytic and other phenomena, the deviation test is invaluable, and certain properties are well known. The number is now so great as to include practically all known bacterial infections; in fact, we may state it as a general law that in such conditions there are formed antistances of the nature of immune bodies which in association with the antigen fix complement. This is especially noted that an antigen body itself is in fact an antistance—immunize—antigen in any case of non-specific fixation of complement. It is quite evident that the method can be applied both for the detection of antigen (given antistances) or of antistances (given antigens), and in my opinion the method is a valid one, and it is so within as wide limits as the methods used in the detection of other antistances, for example, opsonins, agglutinins. It is not only as widely applicable as but of corresponding delicacy, to the other methods, provided that they be properly carried in a quantifiable order. The question is whether a specific antistance is present in the serum, the best method, in my opinion, is to use a fixed quantity of antigen and antistance along with varying amounts of complement in a series of tubes, and if in no less than a mere qualitative test. By such a method, when suitable controls are used, the absorption or fixation of a very minute quantity of complem
the deviation method will be found to be very useful. The method is also of service in considering the etiological relationship of an organism; in this case we have the antitoxin in the serum of the patient, and we test whether the suspected organism will functionate as antigen. The most striking example of this application is the well-known work of Bordet and Gengou with regard to the bacillus of whooping-cough.

The Wassermann reaction, first described by Rondoni, is found to be of practical importance in this respect. It was by the application of the principle of fixation of complement by antigen + antitoxin substance that Wassermann, Neisser, and Bruck discovered the serum reaction of syphilis, this phenomenon now must be considered to be of great importance. Extract of syphilitic organisms was found to be replaceable by extract of normal liver, and this again by solutions or emulsions of substances of comparatively simple and known constitution. The antigen is the spirochaetes, and it is no less certain that it cannot be any product resulting from the action of the latter, as if this were so, it would follow that only extracts of syphilitic tissues could functionate as antigen. It is a matter of regret, in my opinion, that Professor Wassermann in his introduction to this discussion has not drawn this distinction between deviation of complement in various bacterial infections and that in syphilis. In fact, it is only the antigen sensu stricto that, along with the corresponding serum, leads to the fixation of complement by whatever a mixture of substances, organic or inorganic, can act in this way. It is of no great moment that an extract of syphilitic liver is usually more efficient than one of normal liver, and of this another explanation than that he former contains a true syphilitic antigen might be that its content of lipoids is higher. It is known that oleates, glycolipid sodium, cholesterol, etc., and especially combinations of these, can be used to bring out the characteristic reaction with syphilitic serum. Of this fact, the importance of which has been overlooked by him, no real explanation can yet be given. We can only say that we can only say that at present some substance, apparently a modified protein, is peculiar to or present in especially large amount in syphilic serum, and that this, in combination or in association with lipoids, etc., leads to the fixation of a large amount of complement, regardless of the validity of the method, practically all are now agreed, and I would only wish to add that the discovery is, in my opinion, one of the most important in the history of medical science, and one of which the far-reaching influence cannot be yet fully appreciated. I would like, however, in the first place to refer especially to the complexity of the reaction and the various influences which modify it. It is to be noted that the test is a quantitative one, whether or not complement is fixed or not, but one as to the length of time. We are dealing in the reaction with three ingredients, and each of these is a mixture of complex organic substances. The result, that is the amount of complement being fixed, is found to be greater or less in each of the three ingredients, and we may accordingly consider some of these. It was shown by Sachs and Rondoni, and confirmed by Browning and McKenzie, that a turbid emulsion of the extract which acts as antigen, leads, with the syphilic serum, to the fixation of a larger amount of complement than a comparatively clear one; here, manifestly, a purely physical condition plays an important part. These latter observers also found that guinea-pig's complement is deviated in larger amount when it is exposed to the action of the complement for a longer time, though the haemolytic dose may have remained unaltered in the interval. They also showed that individual variations in the faculty of being fixed were met with amongst different samples of guinea-pig's complement, and, in taking as the estimate of the amount of complement, they showed that when several complements were tested, different amounts were deviated by the same mixtures and under the same conditions of treatment. Then, lastly, with regard to the serum to be tested, it is a test the structure of the serum in the fresh state usually deviates (along with the extract) more complement than when it is heated to 55°C. But the same applies to the non-syphilitic serum, and the latter may be dealt with accordingly, so that it is better to use the serum to be tested after heating at 55°C, as the difference between syphilitic and non-syphilitic is in this way more markedly brought out.

From these facts and others of similar nature there follow two conclusions. One of theoretical, the other of practical importance. The first is that in the Wassermann reaction we have to do with a phenomenon of very complex nature, and one whose result may be modified by physical agencies in a way which is not yet understood, and the other is that the degree of similarity with the reaction and scrupulous care in carrying it out are essential for obtaining the best results.

I am not going into the question of the different methods of applying the test, as I understand that this will be dealt with by Dr. Dean. But I may say that in the method containing the extract was found to be replaceable by extract of normal liver, and this again by solutions or emulsions of substances of comparatively simple and known constitution.

We know, for example, that the reactions of extract used in the Wassermann reaction have what we may call an autocomplement effect, as this serum to a slight extent, and the latter is often seen in the serum of normal serum, especially when unheated, and of extract may have a greater effect than the sum of the effects of the components themselves.

Such an effect is often spoken of as non-specific, and the term is convenient, but we can hardly say distinctly whether the specific substance, that is, that of the emulsion, is acting on some new substance, or whether there is merely an increase of a substance normally present. Also, with regard to these non-specific deviations, we cannot say what extent inhibition, or contrasted fixation, plays a part. But it is interesting to note that in these cases the core the condition of the substance concerned is of importance; for example, there is a marked difference according to the readiness or facility of the emulsion.

Another illustration may be quoted from a recently-published paper by Browning, Cruickshank, and McKenzie. Using an extract of ox's liver in cold ethyl acetate, they found that an emulsion of this in salt solution has a very powerful anticomplement effect, which is almost entirely lost when large extent by lecithin when this is added on solution to the extract and the emulsion is then made; on the other hand, when the emulsions are made separately and then mixed, the anticomplement effect remains practically unaltered.

It has been noted by various observers that when human complement—unheated serum—is kept in a sterile condition, its anticomplement action may gradually increase. Browning and McKenzie have found that after two or three months of such a condition, the extract to that used in the Wassermann reaction may antagonize from fifteen to twenty doses of guinea pig's complement, or in other words, such a serum without the addition of any extract would give a positive reaction. This is a remarkable result, and it is equally remarkable that when such a serum is heated for an hour at 55°C this anticomplement effect disappears. These results, which have not yet been published, show that changes taking place spontaneously in a normal serum may result in the power of neutralizing complement and that this property is a very labile one. From facts such as these it is seen how comparatively slight changes in the serum the power of fixing or inhibiting the action of complement may depend.

(2) It is not necessary to consider in detail the fixation of complement by comparatively large organic particles,
In a mixture of a definite slight degree of acidity haemolysis does not occur. The red cells are present sensitized, but they cannot avail themselves of the complement which is also present, and they remain unlaked.

It was shown by Michaelis and Skwirsky that under such conditions the red cells, although not laked, may still be not unaffected by the complement with which they have been in contact. The corpuscles are suspended in an isoelectic solution of sodium phosphate of known acidity, and mixed with complement and the appropriate immune serum. After an interval the mixture is centrifugaled, and the supernatant fluid is removed from the unaltered red cells, and both fluid and red cells neutralized. Even a neutral fluid, and arranging fluids, and red cells remain unaltered. They are fully sensitized, because the addition of fresh complement causes rapid laking.

The failure of haemolysis, then, is evidently due to the fact that the sensitized cells have not taken up the complement when it was offered them.

We should expect, then, to find the complement left in the supernatant fluid, and available in the now neutralized fluid. If, however, we add this neutral fluid to other red cells, sensitized equally, but in the usual way, by the addition of immune serum without complement, no haemolysis occurs. We do not, in fact, find the complement in the supernatant fluid. What, then, has become of it? An explanation, which immediately offers itself, is that the complement has been destroyed by the acidity of the original mixture. This explanation is not the only one. One may add a neutral fluid to the cells from which it was separated by means of the centrifuge while it was acid; haemolysis occurs as readily as if we had added fresh untreated complement. The complement has not been destroyed.

We are then in this case. We have a fluid, the neutralized supernatant fluid, which is unable to laxe sensitized cells in the ordinary way. This fluid, then, must lack an ingredient essential to haemolysis, namely, the complement, or part of it. It can, however, laxe the cells from which it was removed. These cells, then, must contain the missing ingredient. But we know that these cells do not contain the complete complement. Hence we may deduce that the cells contain one fraction of the complement, which I shall call C, and the supernatant fluid another fraction, C'.

The argument may be summarized in this way:

1. Complement + ordinarily sensitized cells = laking. 
Supernatant fluid + ordinarily sensitized cells = no laking. Therefore the supernatant fluid does not contain the complete complement.
2. Treated sensitized cells by themselves = no laking. Therefore these cells do not contain complement.
3. Supernatant fluid + treated sensitized cells = laking. Therefore this mixture contains the complete complement.
4. That is - The treated cells contain one fraction of the complement C, and the supernatant fluid another fraction of the complement C'.

A similar division of complement into two parts was effected some years ago by Ferrata by means of dialysis; but his method is very uncertain and gives very irregular results. One portion came down with the gels which fell out of solution as dialysis progressed, and the other portion remained in solution. Ferrata believed that the globulin part, which corresponds to what we are calling C, is not capable of destroying the sensitized cells. It can be destroyed by half an hour at 55° C, but not by C'. For, if you heat whole complement, you cannot by adding C' restore its activity. It is interesting to note that a C derived from human serum by the acid method described acts at least as well with the C' of guinea-pig serum as does guinea-pig C itself. I am not yet certain whether the C' of human serum acts with the C of the guinea-pig.

Now, in deviation experiments, as ordinarily carried out—that is, with extracts, whether of bacteria or of syphilitic matter, as in the Wassermann reaction—complement is offered to the sensitized cells in a fluid which contains a high concentration of sodium chloride—that is, is hypertonic—whose capacity of taking up the complement may be considerably reduced. There are other methods known, by which we can vitiate these sensitized cells and make them not available to the complement offered to them; but the one to which I wish to direct your attention is that of altering the reaction of the medium in which the haemolytic constituents are mixed.

The mechanism of complement deviation has already been so clearly explained by Professor Wassermann and Professor Muir in the course of this discussion that I need not again describe the process in any detail. Red blood corpuscles when treated or "sensitized" with the serum of an animal immunized to them acquire the property of attracting complement, a substance found in fresh normal serum. This is a property which the red corpuscles share with other cells similarly treated. Most cells, including haemolysins, have the property of attracting complement, and when they are combined with the serum of an animal immunized to them. On this fact is based the whole technique of complement deviation as a means of diagnosis.

Now this attraction of the sensitized red cells for complement may be accentuated or diminished according to circumstances which are within our control. It is a familiar fact that at a low temperature the sensitized red cells are usually unable to take up complement out of the fluid in which they are suspended; they consequently remain unlaked. Similarly, if the serum used is offered to the sensitized cells in a fluid which contains a high concentration of sodium chloride—that is, is hypertonic—their capacity of taking up the complement may be considerably reduced. There are other methods known, by which we can vitiate these sensitized cells and make them not available to the complement offered to them; but the one to which I wish to direct your attention is that of altering the reaction of the medium in which the haemolytic constituents are mixed.
contains C, and only part of the complement has been deviated—namely, C. In deviation experiments, then, what occurs is the removal of C, while O remains free and intact. I have tried this with various forms of deviation, such as the ordinary bacterial extract, the Wassermann reaction, and the precipitin reaction, and always with the same result: only C is deviated. Even in cases of spontaneous deviation, those non-specific deviations of complement which render rigid controls so necessary a part of a reliable Wassermann technique it is not a deviation of the whole complement that occurs, but of only a part, and of always the same part—namely, C.

Whether if one used, instead of bacterial extracts such as to modification introduced by Bordet and Gengou, one would still find only a part of the complement deviated, I cannot yet say. The attraction of red cells sensitized under ordinary conditions is for the whole bacteria, as in the original method of Bordet and Gengou, one would still find only a part of the complement deviated, I cannot yet say. The attraction of red cells sensitized under ordinary conditions is for the whole bacteria, as in the original method of Bordet and Gengou, one would still find only a part of the complement deviated, I cannot yet say.

THE DIAGNOSIS OF SYPHILIS BY THE COMPLEMENTARY DEVIATION METHOD.

I.—Diagnosis at an Early Stage.

DURING the past eighteen months, at the R.N. Hospital, Haslar, I have, for diagnostic purposes, made systematic examinations of the sera of the majority of early venereal cases admitted there, and also of men suffering from other diseases under treatment during this period. In a large hospital situated in a naval port and garrison town the number of venereal cases admitted is naturally large, and I had the advantage of seeing them from both a clinical and laboratory point of view. It was therefore possible to make repeated examinations of any single case, as was frequently done when there was any doubt as to the diagnosis.

For much assistance in this work I have to thank Staff Surgeon Adams, R.N., who was in charge of the cases.

I have generally sent the hospital as soon as possible for treatment, so that valuable opportunities were given for determining how early after infection the presence of the immune body could be detected; this I have endeavored to do. Examinations of sera were made weekly, at the time considered fixed, the tubes numbered and tested under exactly similar conditions throughout. Three methods have almost always been employed with all the sera: (1) Fleming's modification of Hécht's method; (2) The original method with heated sera, but using an alcoholic extract for the antigen; (3) similar methods, but with unheated "test" sera.

A total of 1,165 bloods have been examined, giving the results shown in Table I.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chancroid of face</td>
<td>28th day after infection</td>
<td>42nd day</td>
<td>1 injection.</td>
</tr>
<tr>
<td>2</td>
<td>Syphilis 1</td>
<td>30th day</td>
<td>45th</td>
<td>None.</td>
</tr>
<tr>
<td>3</td>
<td>Chancroid</td>
<td>42nd</td>
<td>49th</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Chancroid</td>
<td>28th, 35th, 42nd days</td>
<td>49th</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Chancroid</td>
<td>13th, 27th days</td>
<td>54th</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Syphilis 1</td>
<td>49th day</td>
<td>56th</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Chancroid</td>
<td>49th</td>
<td>56th</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Chancroid</td>
<td>36th, 42nd, 50th days</td>
<td>57th</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Chancroid</td>
<td>27th day</td>
<td>57th</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Syphilis 1</td>
<td>36th day</td>
<td>60th</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Chancroid</td>
<td>58th</td>
<td>65th</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Chancroid</td>
<td>65th, 72nd, 77th days</td>
<td>84th</td>
<td>3 injections.</td>
</tr>
</tbody>
</table>

The cases of secondary syphilis that gave a negative reaction had almost all had much treatment, and as a rule showed little clinical evidence; in others, when the disease was "latent," negative reactions were most common and generally there was a history of a year's treatment or of a great number of injections. The intermittency of the reaction was frequently seen in cases which had been treated by intramuscular injections. For instance, in a case giving a well-marked positive reaction, after fourteen injections or so, the positive was replaced by a negative one; this continued for some weeks, then the positive reaction again appeared, to be again rendered negative by a third course of seven injections, this disappearance and reappearance making it important to be careful to give no definite opinion from a single examination. Very often men were tested who stated that they had received months of treatment, with twenty to seventy injections, these still giving positive reactions; this is little doubt that in most of these the injections had consisted of the medium without the active principle, the treatment neither giving rise to ptyalism nor cure of the disease.

From the observations here made I conclude that the diagnostic value of the test in early cases is very consider-
able, allowing us after ten weeks following infection, or less, to come to a correct conclusion. In no case of chancre or gonorrhoea in which syphilitic symptoms afterwards developed did I fail to get a positive reaction, and in no uncomplicated non-syphilitic lesion of a positive nature was the result obtained. The usefulness of the test for clearing up the diagnosis in obscure and doubtful late syphilitic lesions has been abundantly proved.

II.—Variation of the Reaction.

During the course of this eighteen months' study for diagnosis, employing the three methods above stated, some differences have been noted in the results obtained.

1. Fleming's method. All the serums were tested about a week after the blood was collected; a spleen extract of syphilitic heart was used as antigen. In many haemolysins of the sheep cells was not complete, but was sufficiently definite to contrast with the "test" tube. Out of 1,100 serums tested, 10 per cent. I found failed to haemolyse the sheep's cells at all; these were not recorded for the specific test. As it is stated that normal blood will at times give a positive reaction by this method, a large number of serums from medical officers and others were examined here; in none of these did I obtain a positive reaction for syphilis. Of the cases for diagnosis, excluding the 10 per cent. which failed to haemolyse at all, the reactions were always correct. In 92 cases (3 per cent.) the positive reaction was obtained earlier than when using the inactivated serum in the ordinary way, as proved by repeated further examinations of the serum, and by the clinical officers of the cases. This method, therefore, seems to be an easy and most useful procedure, acting as an excellent check on other methods employed, and is particularly valuable when very little serum is obtainable.

2. The second or original method, using alcoholic extract of congenital liver for antigen, inactivated "test" serum, sheep's blood cells, standardized haemolytic serum and fresh guinea pig serum. This method was employed in every one of the control tests being carried out each week, before commencing the diagnostic series.

3. A modification of the above suggested by McDonagh, using saline solution. The serums were examined twenty-four or more hours after the blood was obtained; using two rows of test tubes containing saline solution of syphilitic serum and complement in one, and saline solution complement with antigen in the other, to both of which 0.25 cc. of "unheated" "test" serum was added, and after one hour's incubation the necessary haemolytic serum mixed with them, again incubated, and finally read off.

This latter procedure with me gave better results than those obtained when using the heated serum, agreeing generally with the readings by Fleming's method, but without the 10 per cent. of failures; when used with efficient controls it appeared to be the most reliable of the three methods.

VI.—IVY McKENZIE, M.B.

INDIVIDUAL PROPERTIES OF COMPLEMENT AND ORGAN EXTRACT.

A method which is commonly adopted in carrying out the Wassermann reaction depends on the action of a mixture of organ extract and serum on an arbitrarily fixed amount of complement; this amount is, a rule, 0.1 c.c.m. of fresh guinea-pig serum when 1 c.c.m. of a 5 per cent. suspension of sensitized ox red blood corpuscles is added after the serum, organ extract, and complement have been in contact for an hour and a half at 37° C. This procedure of observing the effect on a fixed amount of complement would not be open to objection were it not that the complement itself, and also the extract, are variable factors in the reaction. If 0.1 c.c.m. of fresh guinea-pig's serum represented a constant number of haemolytic doses for the test to be used, and if equal quantities of two different samples of complement behaved similarly in the presence of the same syphilitic serum and organ extract, then the employment of a definite amount of complement in the experiment would be the most simple and satisfactory method of carrying out the test. There is ample evidence, however, to show that considerable variations may manifest themselves in fresh guinea-pig's serum as regards (1) the amount of the minimum haemolytic dose, and (2) the deviation of the complement by organ extract alone, and by serum and organ extract, as measured in haemolytic doses.

The minimum haemolytic dose of the complement containing serum is measured as follows: A dilution of one part of the serum is made with three parts of normal saline, and of this 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 c.c.m. are added to a series of tubes, each containing 1 c.c.m. of a 5 per cent. suspension of ox red blood corpuscles fully sensitized (five times the minimum haemolytic dose of immune body being the amount of maximum sensibilization for guinea-pig's complement) with the homologous immune serum of a rabbit. The tubes are placed at 37° C., and after shaking every quarter of an hour, the result is read at the end of an hour, and the amount of complement in the first tube in which haemolysis is complete is regarded as the "dose." It has been found that if kept in an ice chest the haemolytic dose of complement in guinea-pig's serum may vary very little in three or even in four days; the dose, however, of complement from different samples of serum may exhibit considerable variation; serums of the same age have with the same test corpuscles varied in dosage from 0.0025 c.c.m. to 0.015 c.c.m. Thus, if the complement of 0.01 c.c.m. of guinea-pig's serum must be absorbed before a Wassermann reaction is regarded as positive, this represents in one case forty doses of haemolytic complement and in another case barely seven.

Again, the serum complement may show great variation in deviability, and the variation in this property of the complement manifests itself as regards the inhibiting factor in the extract alone, and also of organ extract along with syphilitic serum. Among the factors which determine such variability, the following must be taken into consideration:

1. The turbidity of the emulsion of organ extract with the saline solution.

2. The age of the complement-containing serum.

3. Individual properties independent of age and of the turbidity of the emulsion of organ extract.

1. The Turbidity of the Emulsion of Organ Extract.—When the alcoholic extract of a minced organ is added to normal saline, the turbidity of the resulting mixture depends on the way in which the mixture is made, even when the same proportions of extract and saline are used. The usual proportion of extract to saline solution is 1:5. If the extract be added rapidly and the mixture shaken up quickly, the resulting mixture or emulsion is comparatively clear; if, however, the extract be floated on the top of the saline solution, and mixture be produced by gradual rotation of the tube, then the resulting emulsion will be comparatively turbid. It has been found that, as a general rule, a turbid emulsion deviates more complement than a clear emulsion, and in the presence of a syphilitic serum comparatively more complement is deviated by a turbid emulsion with serum than by a clear emulsion with the same serum. Table I is taken from an experiment in which this phenomenon was observed.

<table>
<thead>
<tr>
<th>Amount of Complement</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turbid emulsion of organ extract alone 0.6 c.c.m.</td>
<td>0.025 c.c.m. (5 doses) No lysis</td>
</tr>
<tr>
<td>Clear emulsion of organ extract 0.6 c.c.m.</td>
<td>0.03 c.c.m. (6 doses) Trace lysis</td>
</tr>
<tr>
<td>Turbid emulsion of organ extract 0.6 c.c.m. + 0.1 c.c.m. syphilitic serum</td>
<td>0.15 c.c.m. (30 doses) Complete lysis</td>
</tr>
<tr>
<td>Clear emulsion of organ extract 0.6 c.c.m. + 0.1 c.c.m. syphilitic serum</td>
<td>0.15 c.c.m. (30 doses) Complete lysis</td>
</tr>
</tbody>
</table>

Here it is seen that the turbid emulsion alone absorbs more than twice as much complement as the clear emulsion; and in the presence of syphilitic serum almost twice as much complement is absorbed by the turbid emulsion as by the clear emulsion. It is also to be noted that if the result were read according to the single tube method a negative result would be recorded with the clear extract (0.1 c.c.m. complement, almost complete lysis), whereas with the turbid emulsion the result would be positive.
2. The Age of the Complement-containing Serum.—

In general, the age of the complement-containing serum is a factor affecting the deviability of the complement; after standing for upwards of twenty-four hours in the ice chest, although there is little or no diminution in the amount of the haemolytic dose, the complement becomes less deviable, both by the emission of organ extract alone and by the emission along with syphilitic serum. Table II gives an example of the difference between a fresh and an old complement in this respect.

<table>
<thead>
<tr>
<th>Complement I (Four Days Old)</th>
<th>Complement II (Fresh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.096 c.cm. (12 doses)</td>
<td>No lysis</td>
</tr>
<tr>
<td>0.194 c.cm. (24 doses)</td>
<td>Trace of lysis</td>
</tr>
<tr>
<td>0.192 c.cm. (24 doses)</td>
<td>Marked lysis</td>
</tr>
<tr>
<td>0.24 c.cm. (30 doses)</td>
<td>Lysis just complete</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>0.096 c.cm. (12 doses)</td>
<td>No lysis</td>
</tr>
<tr>
<td>0.194 c.cm. (24 doses)</td>
<td>Trace of lysis</td>
</tr>
<tr>
<td>0.192 c.cm. (24 doses)</td>
<td>Marked lysis</td>
</tr>
<tr>
<td>0.24 c.cm. (30 doses)</td>
<td>Lysis just complete</td>
</tr>
</tbody>
</table>

From Table II it is seen that, although the haemolytic dose is identical in the two complements (0.098 c.cm.), the fresh complement (II) is deviated to a greater extent, both by the extract alone and also by the extract along with the syphilitic serum. In combination with the syphilitic serum, 0.195 c.cm. of Complement I (24 doses) gives marked lysis whereas with Complement II 0.3 c.cm. (37 doses) is required to cause the same amount of lysis, and the same ratio holds with respect to the amounts necessary to produce just complete lysis. Accordingly the deviating power of the syphilitic serum in presence of the same amount of the same organ extract is half as great again when measured with a fresh complement as it is with an old complement, although the haemolytic power of both complements is identical.

It is difficult to find a satisfactory explanation for such individual variations in complements. The possibility of conversion of complement into complement-along on standing naturally suggests itself, but the fact that there is not a corresponding fall in haemolytic value makes such an explanation improbable. It might also be supposed that when a complement was only slightly absorbed by an organ extract or by a mixture of organ extract and syphilitic serum this was due to the presence of the guinea pig's serum of a natural immune body for ox corpuscles which was specially suited to act in conjunction with the guinea pig's complement. Browning and the writer have, however, shown that this is not so.

The lytic power of three complement-containing sera was tested as follows:

Complement I (three days old), dose = 0.01 c.cm. With extract alone—2 doses = lysis complete.
Complement II (twenty-four hours old), dose = 0.0075 c.cm. With extract alone—2 doses = lysis complete.
Complement III (fresh), dose = 0.0075 c.cm. With extract alone—2 doses = lysis complete.

Taking 1 c.cm. of a 5 per cent. suspension of washed ox corpuscles, 0.1 c.cm. of Complement I produced only a trace of lysis; 0.1 c.cm. of Complement II produced no lysis; 0.1 c.cm. of Complement III produced almost complete lysis (incubation for one and a quarter hours at 37° C., reading taken next day).

Thus Complement III, though possessing much more natural immune body than I or II, was as great an extent as I or II. Further, the natural immune body was removed from Complement III by treatment with washed ox blood at 0° C. (0.26 c.cm. of the treated serum caused no lysis of 1 c.cm. of ox blood suspension), yet the complement of the treated serum was not inhibited to a greater extent than that of the untreated.

In the same way, experiments were done to test whether the amount of complement absorbed by organ extract together with syphilitic serum was influenced by the amount of natural immune body for the test corpuscles in the complement-containing serum. These same three sera were tested both in the untreated condition, and also after being in contact with washed ox blood at 0° C. Table III gives the result of this experiment.

This experiment shows that in spite of the fact that Serum III contained a considerable amount of immune body for ox corpuscles, still exactly the same number of doses of complement was absorbed from the treated as from the untreated serum. The estimation of the haemolytic value of the treated as compared with the untreated sera showed a slight fall in the former, probably due to dilution. Thus the fresh serum (III) is deviated to a greater extent than the older sera, even although it contained the largest amount of natural immune body from the ox corpuscles. Again, the variation in deviability of different complements does not depend on the procedure by which the complement containing serum is obtained. Certain sera were obtained by immediately defibrinating the blood, and then centrifuging off the serum; in other instances the blood was allowed to clot, and the serum allowed eighteen to twenty-four hours to separate; sera obtained in both ways were used fresh, and kept on ice, and compared under these varying conditions, but no definite and constant difference could be attributed to the variation in the procedure.

3. Properties Independent of Age and Turbidity.—

Specimens of complement-containing serum have occasionally been encountered which were so sensitive to the organ extract emulsion as to render their use in the Wassermann test impossible. In three such instances the extract alone absorbed the complement for 0.05, 0.06, and 0.1 c.cm. of the serum. All the sera which were tested in these experiments, including negative controls, gave positive results. In such cases the results must be discarded, and the experiments re-done to a separate sera; if the results obtained in both ways were used fresh, and kept on ice, and compared under these varying conditions, but no definite and constant difference could be attributed to the variation in the procedure.

Properties of the Emulsion
The properties which come under consideration are:
1. The turbidity of the emulsion of organ extract.
2. The lytic effect of the extract on the test ox corpuscles.

1. Turbidity of the Organ Extract Emulsion.—Reference has already been made to the fact that the turbidity of the emulsion depends on the manner in which the extract and saline solution are mixed. It has also been seen that more complement is deviated by a turbid emulsion with syphilitic serum than by a clear emulsion. As normal sera do not, with a normal complement, deviate complement when a turbid emulsion is used, it is advisable in every instance to employ a turbid emulsion. Syphilitic

<table>
<thead>
<tr>
<th>No. of Doses of Complement</th>
<th>Complement I (Three Days Old)</th>
<th>Complement II (Twenty-Four Hours Old)</th>
<th>Complement III (Fresh)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated.</td>
<td>Treated.</td>
<td>Untreated.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treated.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Untreated.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treated.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treated.</td>
</tr>
</tbody>
</table>

| Syphilitic serum (Fresh)    | 7                               | No lysis                              | No lysis               |
|                            | 10                              | No lysis                              | Distinct trace of lysis|
|                            | 15                              | Very marked trace of lysis            | Lysis just complete    |
|                            | 20                              | Complete lysis                        | Complete lysis         |

| + turbid emulsion of organ extract 0.6 c.cm. | 7 | No lysis | No lysis |
|                                           | 10 | Faint trace of lysis | .. |
|                                           | 15 | Very marked trace of lysis | Trace of lysis |
|                                           | 20 | Complete lysis | Complete lysis |

Thus Complement III, though possessing much more natural immune body than I or II, was as great an extent as I or II. Further, the natural immune body was removed from Complement III by treatment with washed ox blood at 0° C. The treated serum caused no lysis of 1 c.cm. of ox blood suspension, yet the complement of the treated serum was not inhibited to a greater extent than that of the untreated.

In the same way, experiments were done to test whether the amount of complement absorbed by organ extract together with syphilitic serum was influenced by the amount of natural immune body for the test corpuscles in the complement-containing serum. These same three sera were tested both in the untreated condition, and also after being in contact with washed ox blood at 0° C. Table III gives the result of this experiment.

This experiment shows that in spite of the fact that Serum III contained a considerable amount of immune body for ox corpuscles, still exactly the same number of doses of complement was absorbed from the treated as from the untreated serum. The estimation of the haemolytic value of the treated as compared with the untreated sera showed a slight fall in the former, probably due to dilution. Thus the fresh serum (III) is deviated to a greater extent than the older sera, even although it contained the largest amount of natural immune body from the ox corpuscles. Again, the variation in deviability of different complements does not depend on the procedure by which the complement containing serum is obtained. Certain sera were obtained by immediately defibrinating the blood, and then centrifuging off the serum; in other instances the blood was allowed to clot, and the serum allowed eighteen to twenty-four hours to separate; sera obtained in both ways were used fresh, and kept on ice, and compared under these varying conditions, but no definite and constant difference could be attributed to the variation in the procedure.

Properties of the Emulsion
The properties which come under consideration are:
1. The turbidity of the emulsion of organ extract.
2. The lytic effect of the extract on the test ox corpuscles.

1. Turbidity of the Organ Extract Emulsion.—Reference has already been made to the fact that the turbidity of the emulsion depends on the manner in which the extract and saline solution are mixed. It has also been seen that more complement is deviated by a turbid emulsion with syphilitic serum than by a clear emulsion. As normal sera do not, with a normal complement, deviate complement when a turbid emulsion is used, it is advisable in every instance to employ a turbid emulsion. Syphilitic

<table>
<thead>
<tr>
<th>No. of Doses of Complement</th>
<th>Complement I (Three Days Old)</th>
<th>Complement II (Twenty-Four Hours Old)</th>
<th>Complement III (Fresh)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated.</td>
<td>Treated.</td>
<td>Untreated.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treated.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Untreated.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treated.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treated.</td>
</tr>
</tbody>
</table>

| Syphilitic serum (Fresh)    | 7                               | No lysis                              | No lysis               |
|                            | 10                              | No lysis                              | Distinct trace of lysis|
|                            | 15                              | Very marked trace of lysis            | Lysis just complete    |
|                            | 20                              | Complete lysis                        | Complete lysis         |

| + turbid emulsion of organ extract 0.6 c.cm. | 7 | No lysis | No lysis |
|                                           | 10 | Faint trace of lysis | .. |
|                                           | 15 | Very marked trace of lysis | Trace of lysis |
|                                           | 20 | Complete lysis | Complete lysis |
serums which react only weakly with a turbid emulsion do in some cases react negatively with a clear emulsion.

3. The extract—The extract formed by itself does not have a lytic effect on the test corpuscles, otherwise it is impossible to estimate the influence of the extract on the complement. In performing the test an estimation should be made of the amount of complement absorbed by the serum and also by the extract alone. A true determination results only where the amount absorbed by the serum along with the extract exceeds the sum of the amounts absorbed by serum and extract alone. Now, if the extract has a lytic effect on the corpuscles, its effect on the complement is estimated, and hence a control which is indispensable to a thorough test is left out.

Again, under some circumstances, and especially when the single tube method is employed, a lytic extract may with a normal serum give what is apparently a positive reaction. The possibility of a false positive reaction from using a lytic extract should be emphasized in view of the fact that its use has recently been recommended, and in view of the fact that some authorities still persist in advocating the advantages of extract of syphilitic liver in the test. A syphilitic liver is, as a rule, either undergoing autolysis or in a state of fatty degeneration when obtained. An alcoholic extract of such livers is often lytic for ox corpuscles in the amounts used for the Wassermann test; and, if not lytic, it contains substances which may have a lytic effect on ox corpuscles in greater amounts than an extract of normal liver. The apparent advantage of using an extract of syphilitic liver is undoubtedly some extent due to the abnormal amount of fatty substances which it contains. A suspending fluid containing saccharoses and a little antolytic change or fatty degeneration was extracted with alcohol and the extract used in the Wassermann test.

Titrations against an alcoholic extract of guinea-pig's liver showed that it did not, with syphilitic serum, deviate from normal complement, although it may do so with normal serum. Now, it has been found that the lytic property of an alcoholic extract of tissue can be inhibited by the addition of blood serum; and it has been suggested that when a lytic extract is employed in the Wassermann test in which the lytic effect is inhibited by the serum whose lytic properties are being tested. This would, of course, depend on two things—first, on the strength of the lytic effect of the extract, and, secondly, on the inhibiting power of the serum. Extracts are known to vary considerably in their lytic effect on blood corpuscles, and Guin cal has found that sera vary in their inhibitory effect on the same extract. If the serum employed has not a complete inhibitory effect on the lytic property of the extract, then part of the guinea-pig's complement-containing serum is lost in the inhibitory process, and the complement is thus destroyed. In this way, with the extract of a certain lytic strength, a negative serum may appear to give a positive Wassermann reaction.

With these variations in the complement and organ extract, attention is drawn to the following precautions:

1. It is advisable to estimate the deviation in terms of the number of haemolytic doses absorbed by serum and organ extract, in addition to the number absorbed by serum and organ extract alone.
2. The complement-containing serum should be allowed to stand twelve to twenty-four hours before use, and should not be more than three days old.
3. The extract obtained from the employment of a complement which is specially sensitive to the organ extract emulsion should be discarded.
4. The organ extract emulsion should be turbid, and shall not be lytic for the test corpuscles.
5. Negative and positive sera should be used as controls in every experiment.

VII.—H. R. DEAN, Lister Institute, London.

COMPARISON OF THE ORIGINAL WASSERMANN METHOD WITH SOME OF ITS MODIFICATIONS.

Relative Value of Different Methods.

One of the most interesting and, as the most difficult question in connexion with the Wassermann reaction is that which deals with the relative value of the various methods of preparing the extract. So long as we remain ignorant of the actual nature of the antigen it seems hardly possible to deal with the question from a quantitatively standpoint.

Supposing that we compare two extracts—the one made from the liver of a congenital syphilis, the other from a normal guinea-pig's heart, we are not so much comparing the methods of preparation as the relative richness in antigens of two chance specimens. One guinea-pig's heart will differ considerably from another, and two specimens of liver will be found to be equally rich in antigen. It seems to me impossible at the present time to make a quantitative comparison between extracts of normal and syphilitic organs. On the other hand, it is possible to draw some sort of conclusion by testing a number of sera against two extracts and noting the results.

I have records of 195 cases in which I have compared an alcoholic extract of normal guinea-pig's heart and a saline extract of syphilitic livers. Of these 195 a positive result was obtained with the liver extract in 31 cases and with the extract made from normal guinea-pig's heart in 22 cases. In 1 case only was a positive result obtained with the alcoholic, but not with the watery extract.

The majority of the 195 cases which I have quoted formed part of a series of cases of children who were examined with a view to establishing a diagnosis of congenital syphilis, and the large number of the cases which I have been able to work with other evidence of syphilis. On the other hand, I have never been able to demonstrate any difference between extracts made from normal and syphilitic organs in testing sera from cases of obvious secondary and tertiary syphilis. The class of case in which I have found a definite difference between the extracts made from normal and syphilitic organs was one in which I have been able to demonstrate that the serum diagnosis could not be confirmed by the Wassermann test. Although I am inclined to the opinion that the more material from syphilitic organs is obtained, the more results are obtained, it is not possible to exclude the view that it is actually too delicate. On the whole, I am inclined to think that in cases of congenital syphilis, and in those cases in which many years have elapsed since the supposed infection, the extracts made from normal and syphilitic organs should be preferred. The question is very important from a practical standpoint, because it is only in latent and obscure cases that there is any real necessity for the serum reaction as an aid to diagnosis.

Another very important point with regard to the extract is its titration and standardization. It is absolutely essential to titrate a new extract in falling doses against several authenticated positive and negative sera. Extracts vary considerably in strength and properties, and it is not safe to use a fixed dose of an extract unless it is shown by titration that it is appropriate to the particular extract in use. It is necessary to determine the dose in which an extract without addition of serum will absorb complement, the dose in which complement is absorbed in the presence of normal serum, and the dose which, in the presence of positive serum, will bind the complement. A good extract is one which shows a large difference in the dose necessary for the fixation of complement in the presence of syphilitic and normal serum. An extract dose which can be relied on to give a positive reaction with a syphilitic serum, and which at the same time is less than half the amount which binds complement in the presence of normal serum.

Extracts should be tested before coming into use, and at frequent intervals, to guard against deterioration in the qualities of the extract, which deterioration is in the case of the watery extracts often very unpleasantly sudden. Adequate standardization of the extract can, I think, be only obtained by carrying out the use of a haemolytic system which can itself be standardized, that is to say, there must be added to each tube a known definite amount of haemolytic serum and a known amount of complement. A titration which relied on the normal amboceptor and normal complement present in the test tube would show little evidence which would be of service in performing experiments with other sera.
One of the great objections to all modifications which make use of the normal complement is that the worker can never determine the presence of the particular dose of extract to absorb complement in the presence of normal serum. In several of the modifications commonly used reliance is placed on the normal amboceptor for sheep's corpuscles which is present in human blood. I examined 50 sera to ascertain the content of normal haemolytic amboceptor for sheep cells.

The tubes were made up to a bulk of 2.5 c.c.m., and contained 0.5 c.c.m. of a 1 in 20 suspension of sheep's corpuscles, and 0-1 in 10 dilution of the guineas' serum. The tubes were incubated for two hours at 37° C. The human sera were inactivated and used in various doses. Of the 50 sera examined the only case was present in 2 cases.

Thus 0.1 c.c.m. serum was found to contain in one case twenty minimal haemolytic doses, in 17 cases eight, in 3 cases four, and in 3 cases two, in 4 cases one, in 7 cases less than one dose, and in 2 cases no haemolysis occurred.

The amount of normal haemolytic amboceptor is a variable factor, even in the original method; but in the control experiment the only question which arises concerns the possible presence of excess of amboceptor. In the case which I mentioned in which 0.1 c.c.m. of serum contained twenty minimal haemolytic doses, a marked positive result was obtained without any particular precautions being observed.

To further test the effect of excess of haemolysin on the reaction, the following procedure was employed:

Several sera, known to give a positive reaction, were absorbed with sheep's corpuscles in the cold. The normal amboceptor for sheep's corpuscles was in this case absorbed and its removal was proved by experiment.

Since the serum in which the haemolytic amboceptor had been removed were then titrated with falling doses of extract, and a similar series of tubes were put containing extract and of the untreated sera. It was found that the intensity of the reaction had been but little influenced by the removal of the normal amboceptor—this is to say, the untreated serum gave almost as marked a reaction as the serum from which normal amboceptor had been removed. A sufficient dose of a haemolytic serum (rabbit v. sheep) was, of course, added to each series of tubes.

The result indicates that a positive reaction is in no great danger of being masked by excess of the normal amboceptor.

In Bauer's and Hecht's method reliance is placed on the normal amboceptor. The danger lies, I think, not in the few cases in which the amboceptor is completely absent, but in those cases where just sufficient is present in the control tube, which contains serum alone, to produce complete haemolysis. In such cases the presence of extract will just upset the balance, and by the absorption of a certain amount of complement a positive result will be simulated. The presence of a known normal and a known syphilitic serum with extract does not afford adequate controls, for the control negative serum may be an unusually powerful haemolytic factor in the system.

The normal complement is not so variable; nevertheless, considerable variations do occur. In an examination of 90 sera I found haemolytic complement apparently absent in 2 cases, and in 3 cases the amount present was very small. I was able to test 37 sera by Wassermann's method, and by the method of M. Stern. Of these, 14 were positive by the original method, and 23 by Stern's method. The 37 sera were also tested for presence and amount of amboceptor. Of the 6 cases which were positive by Stern's method and negative by Wassermann's method, 4 could be shown to be unusually deficient in complement.

Another factor in producing a relatively high percentage of mistakes is probably the use of unheated serum. Sachs and Boas have shown that the serum of a dog with a suspected syphilis will give a positive reaction in greater dilution before it is heated. Boas has further shown that during progress of mercurial treatment a positive result can be demonstrated for a longer period by the use of unheated serum. Nevertheless, evidence has accumulated that the serum of non-syphilitics is apt to give a positive reaction.

It seems to me that the original method is safer for the reason that it permits of the standardization of the reagents and the putting up of adequate controls. The reaction is a quantitative one, and we consider a serum to give a positive result which gives in the presence of extract a very much more marked fixation of complement than a normal serum. We may arrive at our result only if it has suggested by varying the dose of complement or by the previous titration of the extract with a view to the determination of the dose which fixes complement with syphilitic but not with normal serum. The variations in the complement content of the guinea-pig serum produce an adequate met by the routine titration of the haemolytic system before the commencement of the actual test, and the use of a dose of haemolysin which, acting in conjunction with a particular complement-containing serum, will give a result from these three standard doses, the variations in the complement are. I think, in practice adequately compensated by varying the dose of the haemolytic amboceptor.

Reliable results can only be obtained by employing a method which allows of a standardization of the reagents employed, and in which controls can be used which disclose all possible sources of error; and although various modifications have been employed with considerable success, the evidence at our disposal is invariably in favour of the original method.

VIII.—L. W. Harrison, Captain, R.A.M.C.

THE GUIDANCE AFFORDED BY COMPLEMENT FIXATION METHODS.

Syphilis is, unfortunately, a very important disease in the army and the value of the Wassermann serum, from the point of view of diagnosis and of regulating treatment, has been recognized by the Army Medical authorities for some time. It has been my privilege to conduct an investigation at the Military Hospital, Rochester Row, into the value of the reaction from these standpoint, and, although the investigation is as yet in its infancy, some points have arisen which may be of interest to you.

As you are probably aware, when a soldier is found to be suffering from syphilis all the particulars relating to his complaint and its treatment are entered on a case-sheet, which is attached to his other documents and accompanies him wherever he goes. In this way he is kept continually under supervision while he is serving with the colours, and his treatment pursues, as far as possible, a perfectly regular course.

The advantage, from the point of view of the Wassermann test, is that at the same time as a syphilitic soldier's blood is tested for this reaction a perfect record of his clinical progress and his treatment is available, and it is possible to correlate all the particulars with the result of the test.

As it was necessary to ascertain if the two years' treatment, which is customary in the army, was sufficient as judged by the Wassermann reaction, I had to search for the most delicate and yet reliable method of conducting the test. As a result of a number of experiments, I found that by heating the serum at 55° C. for ten minutes only (which is sufficient to destroy its natural complement), as compared with the unheated serum, a very considerable gain in the percentage of positive reactions in these well-treated cases was obtained, though the difference might not be so apparent in the untreated and early cases. At the same time, the uncertain element introduced by omitting to destroy the natural complement was eliminated, and, while maintaining the reliability of the original technique, the test approached such modifications as Hecht's and Stern's in delicacy.

I have found it an advantage in estimating the effect of treatment to test the serum in a roughly quantitative manner by putting it up with two different amounts of complement (on the lines suggested by McKenzie), one corresponding to 0.1 c.c.m. fresh guinea-pig serum, on
which I standardize my reagents, and the other corresponding to 0.2 c.c.m.

I have obtained the following results, which illustrate the effect of treatment on the reaction:

<table>
<thead>
<tr>
<th>Untreated syphilis, including primary cases from the fifteenth day</th>
<th>Positive 0.2% Complete Complement.</th>
<th>Positive 0.1% Complete Complement.</th>
<th>Total Positive</th>
<th>Total Negative</th>
<th>Percentage Positive.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilitic cases:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 1 course of treatment</td>
<td>17</td>
<td>29</td>
<td>46</td>
<td>9</td>
<td>83.6</td>
</tr>
<tr>
<td>After 2 courses</td>
<td>24</td>
<td>14</td>
<td>38</td>
<td>11</td>
<td>77.5</td>
</tr>
<tr>
<td>After 3 courses</td>
<td>8</td>
<td>19</td>
<td>27</td>
<td>15</td>
<td>64.5</td>
</tr>
<tr>
<td>After 4 courses</td>
<td>3</td>
<td>14</td>
<td>17</td>
<td>13</td>
<td>53.0</td>
</tr>
<tr>
<td>After 5 courses</td>
<td>4</td>
<td>13</td>
<td>17</td>
<td>17</td>
<td>50.0</td>
</tr>
<tr>
<td>After 6 courses</td>
<td>3</td>
<td>9</td>
<td>6</td>
<td>9</td>
<td>34.6</td>
</tr>
<tr>
<td>After 7 courses</td>
<td>5</td>
<td>11</td>
<td>16</td>
<td>13</td>
<td>55.0</td>
</tr>
<tr>
<td>After 8 or more cases</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>11</td>
<td>21.5</td>
</tr>
</tbody>
</table>

You will notice that as treatment progressed there was a marked falling off in the percentage of positive reactions with the double amount of complement (0.2 c.c.m.) as well as in the totals which gave the reaction in all.

The table shows an increase in the percentage of positive reactions given by sera of cases which had received seven courses as compared with those which had received six, and I think that this apparent anomaly is to be explained by the fact that the cases under this heading included a number of men who had passed to the Reserve but had voluntarily persevered with treatment on account of persisting symptoms.

Taking a positive reaction as a certain indication to persever with the treatment, it appears clear that two years is not a sufficiently long period to lay down as a routine measure for the treatment of this disease.

Regarding the modifications which have been introduced on the claims of greater simplicity and that of delicacy, such as Hecht's and Stern's, I have practical experience only of Stern's, though I have seen a considerable number of tests conducted by Colonel Birt, using Fleming's modification of Hecht's technique.

I think that, in conjunction with the employment of one of these modifications is a considerable advantage on account of its superior delicacy when estimating the effect of treatment, but for diagnostic purposes all of them suffer from an inevitable objection of which I have practical experience. If a serum has been heated not entirely complement, the extract may cause absorption of the medium of complement in it, even though it be a normal serum, and yet this small amount of complement, unhampered by the extract, may be sufficient to cause perfect haemolysis in the control tube.

I do not believe this test can be carried out by the practitioner in the course of his other duties, and being in any case a laboratory procedure, I do not think that the modifications which have been introduced give a sufficient gain in simplicity to justify their substitution for a technique based on the original lines of keeping the reagents for the test as far as possible constant, and one therefore under more perfect control.

LECHITHIN AND CHOLESTERIN AS REAGENTS FOR THE DETECTION OF SYPHILITIC SERUMS.

It is now generally agreed that for the detection of syphilitic sera by the complementation method the most suitable form of antigen is an alcoholic extract tissue. The fact that the active substances were soluble in alcohol led to the supposition that they were of lipid nature. Accordingly a number of such lipid bodies have been introduced in the Wassermann reaction, and also mixtures of these compounds, such as that of Sachs and Rondoni, have been employed; but, so far, the crude extract has proved superior to any of these combinations. As the question of the properties of alcoholic extracts in regard to the sulphur reaction has already been dealt with (McKenzie) I shall consider the action of the two tissue constituents—lecithin and cholesterin—which we have found to act as very delicate reagents for the determination of changes in serum due to syphilitic infection (Browning, Cruickshank, and McKenzie).

Lecithin by itself has only a moderate capacity for absorbing complement in the presence of syphilitic serum, as many workers have found.

We have used lecithin prepared from the dried residue of a crude alcoholic extract of fresh ox liver.

The residue is extracted with ethyl acetate at 60° C. and the solution filtered. The portion which falls out in the cold is dissolved in ether and precipitated with acetone (the ordinary commercial "pure" reagents serve for this purpose); the process of dissolving in ether and precipitating with acetone is repeated twice again, and finally the precipitate is treated with absolute alcohol in a mortar till the soluble portion has been extracted. In this way we have obtained lecithin, of which the lytic dose for ox blood corpuscles is three to six hundred times the amount which causes lysis in the presence of cobra venom. For the Wassermann test 1 volume of 0.75 per cent. lecithin in alcohol is diluted with 7 volumes of salt solution so as to make the emulsion as turbid as possible. A series of tubes containing 0.1 c.c.m. of this emulsion mixed with 0.05 c.c.m. of the serum, previously heated at 57° C. for thirty minutes, are then used to estimate the power of absorbing the complement of guinea-pig's serum. After the mixture of emulsion, patient's serum, and complement has stood for one and a half hours at 37° C., the test blood suspension (0.1 c.c.m. of 5 per cent. was heated with five doses of immune body from the rabbit—is added to each tube. After one and a quarter hours' further incubation the tubes are set at room temperature and the result read next day.

We have found that in the presence of powerful syphilitic sera a very considerable amount of complement may be absorbed by the lecithin; on the other hand, with less powerful sera, no more complement is absorbed than in the presence of the sera of normal individuals. When cholesterin is added to such an alcoholic solution of lecithin a considerable amount is dissolved.

To the 0.75 per cent. alcoholic lecithin solution cholesterin is added in excess, and the mixture is allowed to stand for 18 hours at room temperature. As saturation occurs only slowly at room temperature, the mixture should be allowed to stand for a week before the clear fluid is used. In dilution with 7 volumes of salt solution, as in the case of the lecithin, a uniform emulsion results.

In the presence of syphilitic serum the lecithin-cholesterin emulsion leads to the absorption of more complement than is absorbed by the equivalent amount of lecithin in emulsion in the absence of cholesterin. With negatively reacting sera the emulsions of lecithin and of lecithin-cholesterin practically always absorb the same amount of complement. Thus, with 70 negative sera, the lecithin-cholesterin emulsion only once absorbed as much as one dose more than the lecithin emulsion. Further, the inhibitory effects of the emulsions of lecithin and of lecithin-cholesterin by themselves on complement are almost always equal. In 42 experiments the amount of complement absorbed by lecithin-cholesterin emulsion only twice amounted to more than the amount absorbed by the lecithin emulsion.

In this respect the lecithin and the lecithin-cholesterin behave much more uniformly with different specimens of complement than crude extract does. The following table

<table>
<thead>
<tr>
<th>No. of Experiment</th>
<th>Lecithin</th>
<th>Lecithin-Cholesterin</th>
<th>Crude Extract</th>
<th>Liver.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.3</td>
<td>1</td>
<td>3.5+</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>1.5</td>
<td>1.5</td>
<td>6+</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>VIII</td>
<td>2.5</td>
<td>2.5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>IX</td>
<td>1.5</td>
<td>1.5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>1.5</td>
<td>1.5</td>
<td>2.5</td>
<td></td>
</tr>
</tbody>
</table>
shows the number of doses of complement absorbed by emulsions of lecithin, lecithin-cholesterin, and crude extract of fresh ox liver respectively when tested simultaneously in a series of experiments.

The absolute amount of complement fixed by lecithin-cholesterin emulsion along with syphilitic serum is in most cases somewhat inferior to that absorbed with crude extract, but at the same time the crude extract by itself usually absorbs more complement than lecithin-cholesterin. We have found, however, that certain serums repeatedly absorbed more complement with lecithin-cholesterin than with crude extract, so that the result is independent of the complement used. This fact serves to emphasize how complicated the nature of the processes involved in the Wassermann reaction must be.

In the ordinary method of carrying out the test with an alcoholic organ extract the criterion of a positive reaction is, of course, the absolute amount of complement absorbed (measured in haemolytic doses) as compared with the amounts absorbed in the controls. Now it has been observed that the reaction in the complement is abnormally susceptible to the inhibitory action of the extract or the heated serum. Along with such deplorable complements a normal serum may cause the absorption of much more complement than the sum of the amounts absorbed by serum and emulsion separately, so that the reaction is apparently positive. For this reason McKenzie and others have suggested that where the extract alone absorbs more than four doses of complement the results should be rejected. In a series of forty experiments made by Dr. Gilmour and myself the results were on ten occasions vitiated by the use of complements which were abnormally deplorable so far as the crude extract was concerned. On the other hand, with the lecithin-cholesterin method, where the difference of the amounts of complement absorbed in the two tests is taken into account, we have found that with highly deplorable complements two serums absorbed the same amount of complement in the presence of lecithin; but, on the addition of cholesterin, the one absorbed much more complement, thus showing its syphilitic character, whereas the other from a non-syphilitic subject deviated exactly the same amount as without cholesterin. The advantage of the use of lecithin and lecithin-cholesterin is thus apparent. The following is an illustrative experiment:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilitic</td>
<td>8</td>
<td>24</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>9</td>
<td>9</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

Controls: Lecithin alone absorbed 3 doses; lecithin-cholesterin alone absorbed 2 doses; crude extract alone absorbed 2 doses; ordinary syphilitic serum alone absorbed 2 doses; normal serum alone absorbed 3 doses.

It is notable that the proportional increase in the amount of complement absorbed under the influence of lecithin is often especially marked in the case of weakly positive serums. Thus, in the following experiment a strong and weak positive serums were tested at the same time:

<table>
<thead>
<tr>
<th>Serum</th>
<th>Serums:</th>
<th>Lechthin.</th>
<th>Lecithin-cholesterin.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak syphilitic</td>
<td>3</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Powerful syphilitic</td>
<td>23</td>
<td>35</td>
<td></td>
</tr>
</tbody>
</table>

Controls: Lecithin alone absorbed 1 dose; lecithin-cholesterin alone absorbed 1 dose; serums alone absorbed 1 dose.

With regard to the mechanism of the specific action of lecithin-cholesterin with syphilitic serums, little can be said of it. Along with Dr. Cruickshank it has been noticed that the reaction is of a more complex nature; together with lecithin and syphilitic serum, and we have found that they all possess in some degree the power of increasing the amount of complement absorbed. Thus it would appear that the intact nature of the alcoholic hydroxy group of cholesterin is not absolutely essential for the reaction.

REFERENCES.

X.—J. O. Wakelin Barratt, M.D., D.Sc.

COMPLEMENT DEVIATION IN RELATION TO CARCINOMA.

In relation to carcinomata the production of antibodies has been studied by many observers by means of selective absorption and of complement deviation, but progress in this direction has been limited by the lack of specificity which the antibodies obtained exhibited. Although the second of these methods is not as yet available for purposes of diagnosis in carcinomata, and it is doubtful if it will be so in the future, nevertheless, it may at this juncture be of interest in connexion with the Wassermann reaction to allude briefly to the occurrence, in the serum of the subjects of carcinomata, of substances which, when employed in conjunction with tumour extract, produce complement deviation.

In connexion with human carcinomata the method of complement deviation has been employed by Bazzi with negative result and, more recently, by Luidke with positive result.

In relation to mouse carcinomata and also to human carcinomata I have been able to make some observations, which may here be briefly described. Full details being reserved for a paper shortly to be published. In these experiments an attempt was made with the aid of the inactivated serum of the subject of carcinomata, in conjunction with an extract of the corresponding tumour, to produce complement deviation. A series of observations made upon mouse carcinomata presents the advantage of being homogeneous in character, representing the result in different animals of the same type of pathological process, the same strain of tumour being employed throughout the series. On the other hand a similar series of observations
Memoranda: MEDICAL, SURGICAL, OBSTETRICAL.

A CASE OF FENASE.

PRIVATE G. C., South Lancashire Regiment, was admitted into the Station Hospital, Jubbulpore, C.P., in October, 1906. He complained of severe pains in the nasal cavity, chiefly referred to the posterior nares, a sense of discom- fort, and at times severe pain in the frontal region. There was a muco-purulent discharge from the anterior nares, and the temperature was 102.4°. He had been on outpost duty ten days before, during the cold weather manoeuvres between Jubbulpore and Sangor, and contracted a "cold" in the tent he was accustomed to sleep in.

He gave a distinct history of the actual morning of the attack. The progress of the case was unsatisfactory in the early stages; the pains increased, the temperature was of a remittent type, varying from 101.2° to 103°; the discharge extended to the posterior nares, and that from the anterior nares became watery. The larynx was normal throughout. On the morning of the 13th a bulging was observed in the soft palate, which gradually increased, very painful from the beginning. A linear incision in the middle line, previously painted with a 10 per cent. solution of cocaine, was followed by a discharge of pus; the latter was offensive in nature, mixed with mucus, and was afterwards confirmed as the larva of the screw-worm. The progress of the case was slow. Inhalinges of chloroform failed to destroy the larvae. Injections of equal parts of chloroform and water, and the oil of potine, were also used; the latter, in my opinion, is much more satisfactory, and eventually quite successful. The temperature rapidly fell, and there was no extension of inflammation into any of the neighbouring sinuses.

I am indebted to Lieutenant-Colonel Morris, officer commanding Station Hospital at Jubbulpore, for the photograph from which the illustration here given is reproduced.

J. GERALD BERNE,
Naples.
Captain, R.A.M.C. (ret.).

Reports on MEDICAL AND SURGICAL PRACTICE IN THE HOSPITALS AND ASYLUMS OF THE BRITISH EMPIRE.

BRISTOL GENERAL HOSPITAL.

A CASE OF DISELOCATION OF THE LOWER CERVICAL SPINE.

(Reported by DAVID ROBERTSON, M.B., Ch.B., House-Surgeon.)

The patient in the following case, a man aged 29, was admitted under the care of Mr. E. W. Hey Groves at 4 p.m. on August 2nd. The history given was that at about 6 p.m. on the previous day he was swinging in a swing-boat. He stood up and tried to catch the rope by which the boat was actuated, and, missing his hold, he fell forward out of the boat, his toes catching in the opposite seat. He landed on his right shoulder and hands, and somersaulted on to his back. On striking the ground he was conscious that "something in his neck cracked," and that he was paralyzed from the neck upwards.

State on Admission.—Then, paralysis of legs, abdominal and organic muscles; partial paralysis of the deltoids, adduction at the shoulder-joint being imperfect, and all the other arm movements were lost. Sensibility to touch, cold, and sharp pain was lost below the level of the second rib in front, and the spine of the scapula behind, and in the arms below the centre of the deltoid. Pupils were equal and narrowly contracted; corneal reflexes did not dilate in the shade. With regard to organic reflexes, he had incontinence of faeces and retention of urine. Respiration was jerky and diaphoretic. Examination of the muscles of the head, neck, and shoulders, showed the trapezius, supinator, and biceps to be absent on both sides. There was no giddiness, sensation. He could rotate his head slightly without pain. Any marked movement caused pain in the cervical region and behind. Palpation of neck failed to reveal any projection. There was marked tenderness at the sixth cervical spine.

Course.—By the morning of August 3rd he had total paralysis of the arms, and the arm jerks were absent on both sides. With the possibility of there being extradural or subdural haemorrhage, Mr. Groves operated in the afternoon, under chloroform. On removing the laminae of the fifth and sixth cervical vertebrae the dura mater at once bulged backwards. There was pulsation. On incising the dura mater a softened area of cord was apparent; no fracture of the spine detected; fifth cervical vertebra thought to be abnormally mobile.

Result.—In the morning of August 4th he seemed to be in much the same condition as the previous day, with complains of incontinence of urine and faeces, and sleepwalking in the hands. Later had intense pain all over, requiring morphine. At 12.15 a.m. on August 5th he complained of sharpness of breath; fifteen minutes later convulsions ceased; the heart continued beating for eight to ten minutes.