CASE IX.

M. M., aged 11, seen in consultation with Dr. Barnes of Fails-worth on April 28th, 1918. She had been ill for about fourteen days with pain in the head and mild pyrexia, about 100° F. The pain was mostly about the back of the head and neck, and there had been distinct stiffness of the muscles at the back of the neck. She had vomited once, and the tongue was dirty. There had been a good deal of general malaise, but when I saw her she was better and was sitting up in bed reading and anxious to be allowed up. There was no paralysis of any of the face muscles, nor any complaint of double vision, but she had well-marked double optic neuritis. On May 5th Dr. Barnes informed me that she had since complained of double vision, though there was no recognizable ocular paralysis. The optic neuritis is now beginning to clear up.

CASE X.

Mrs. W., aged 61, seen in consultation with Dr. McLean of Harpurhey on March 20th, 1918. She had had gradually progressive asthenia for two or three weeks with slow mentality and forgetfulness, and slight continued pyrexia. It was difficult to get her to answer any questions about herself, though after I had been with her for a time she roused up to a certain extent, and took some interest in my visit. There was no paralysis of cranial nerves then, and no optic neuritis, but she subsequently developed facial paralysis. She gradually got worse, and sank into a more and more confused and asthenic condition, and died about a fortnight later.

CASE XI.

Mrs. P., aged 62, also seen in consultation with Dr. McLean of Harpurhey on April 3rd, 1918. She had had symptoms of arterio-sclerosis—recurrent headache and dizzy attacks—for two or three years past. For some days before I saw her she had been suffering from headache, and had been dull and confused, and the day before sudden ptosis of the left eye had developed, and she had diplopia, due to fairly complete paralysis of all the straight muscles of the left eye. She complained of a good deal of pain and headache above the eye, made worse on examining for optic neuritis, which was absent. The urine was free from albumin. She had gradually improved, but on May 5th there was still some drooping of the left lid and diplopia.

With the exception of Mrs. W. (Case x) and of G. T. (Case III), the general symptoms have been comparatively slight, certainly not of the severity suggested by Professor Hall in his brief note of the cases he has seen in Sheffield. It would appear as if most of the cases I have seen were of a subacute type.

I have had some doubts as to whether the three cases of simple unilateral facial paralysis should be included, since they differed in no way from the sporadic cases with no ascertainable cause that are of fairly common occurrence. In view of their unusual frequency, however, and of the belief which I know is held by some physicians that facial paralysis is not uncommonly an expression of polio-encephalitis, I have thought well to retain them.

I have some doubts also of the exact nature of the two following fatal cases. In neither of them was there any cranial nerve paralysis, but the progressive asthenia and drowsiness, advancing to coma, without definite evidence of meningitis, have inclined me to believe that they fall into the same category. I may say that I have during the period come across (but not had under my own care) several cases of similar type, some of which have ended fatally, whilst others have got well after being in apparently a dying condition for some days.

CASE XII.

Mrs. N., aged 28, seen with Dr. Kenny of Pendleton on February 16th, 1918. She had started about February 12th with symptoms suggestive of a mild influenzal attack and a temperature of 100° F. On February 13th she was better, but on February 14th complained of headache and began to be drowsy, and when I saw her she was unconscious, though she showed that she could just feel firm nipping of the skin between one's finger nails. Her pulse was fast (120), breathing not faster than normal. There was nothing abnormal to be discovered in the heart, lungs, or elsewhere. The urine was normal. There was no paralysis, and no optic neuritis. Her state of coma steadily deepened, and she died a few days later.

CASE XIII.

L. W., aged 22, seen with Dr. Barnes of Fails-worth on March 19th, 1918. She had been ill for about a week, having started with chest symptoms—cough, rapid breathing, and pyrexia. For two or three days before I saw her she had been getting progressively more drowsy and weaker, and finally could not be roused at all. The urine was normal. There was no paralysis, no convulsions or twitching, but as I suspected tuberculous meningitis I took her into the Royal Infirmary under my care. Lumbar puncture yielded clear fluid under moderate pressure, which did not yield any pathological organisms on culture, and did not show the presence of tubercle bacilli or *Diplococcus intracellularis*. Her temperature remained high and

her coma gradually deepened till she died, two days later. The autopsy revealed purulent bronchitis and acute pulmonary emphysema, but there was no meningitis nor any recognizable naked-eye abnormality of the brain or meninges.

NEPHELOMETRIC ESTIMATION OF QUININE
IN BLOOD AND URINE.

BY

I. J. LIPKIN, M.B., CH.B., M.R.C.S., L.R.C.P.,

JOHNSTON FELLOW IN BIOCHEMISTRY,

AND

W. RAMSDEN, M.A., D.M.,

PROFESSOR OF BIOCHEMISTRY IN THE UNIVERSITY OF LIVERPOOL.

THE methods recorded below were devised, amongst others, in the course of work undertaken at the request of the Medical Research Committee. They are published at once in the hope that they will be useful to others who are investigating the treatment of malaria by quinine. A fuller account of these and other methods will appear in the *Annals of Tropical Medicine and Parasitology*.

By the following procedure estimates of small fractions of a milligram of quinine can be made with an error of less than 5 per cent. It has been found invaluable in dealing with such small amounts of the alkaloid as are contained in 5 c.cm. of human blood, when no other methods would have the necessary delicacy. It has also, by reason of its rapidity, proved very useful for approximate estimations of quinine in urine. Its success is due (1) to the great delicacy given to the Tanret turbidity test by using purified ether and by dissolving the quinine in saturated ammonium sulphate solution; (2) to the observation that comparisons of the opalescences of two suspensions can be made with greatly increased accuracy when the tubes are illuminated by a band of light admitted through a narrow adjustable slit.

Estimation of Quinine in Blood.

Five to ten cubic centimetres of blood are removed from the patient by a syringe, ejected at once into a weighed flask containing 2.5 to 5 grams of solid ammonium sulphate and 10 c.cm. of a saturated aqueous solution of this salt containing 0.6 per cent. H_2SO_4 , and the flask is re-weighed. Boil for two minutes, shaking all the time, and pour off the liquid portion while hot into a small Gooch crucible under suction. Catch the filtrate in a test tube and transfer it to a 50 c.cm. stoppered cylinder. Add 10 c.cm. more acidulated ammonium sulphate solution to the original flask, boil and filter as before. Wash the residue on the crucible twice with the hot filtrate before adding it to the original filtrate. Repeat the boilings with three further lots of acidulated ammonium sulphate solution, on each occasion washing the residue on the crucible two or three times. Cool the filtrate, alkalize it with strong ammonia and extract with four 7 c.cm. lots of purified ether (*v. infra*), removing each lot as it separates, and transferring it to one of a special series of test tubes gauged for nephelometry. Evaporate off the ether on a hot water bath, taking care to avoid loss by spurring—the tube now contains the whole of the quinine of the original blood. Control experiments with normal blood have shown that it contains no substance giving turbidity with Tanret's reagent. The test tubes are of thin-walled colourless glass, and have a diameter of about 13 mm. They are carefully selected so as to be equal in calibre in the lowest two centimetres of the cylindrical portion, this equality being ascertained by means of a suitably thick piece of glass tubing surrounded at its lower end by a half-centimetre length of thick black rubber tubing.

The nephelometer consists essentially of a well made dull-black parallel ruler, the lower bar of which is screwed horizontally to a wooden supporting open framework (for example, a photographic half-plate printing frame), which bears also a small shelf level with the lower edge of the ruler. By means of vertical cardboard screens light is blocked off from all but a middle portion of the slit two test tubes wide. The light should be strong. Between it and the slit is interposed a piece of opal glass or white semi-transparent paper. The ends of the test tubes rest on the transverse shelf referred to above, their sides are

kept in close contact with the face of the ruler by means of rubber bands or brass springs screwed to the ruler or the frame.

Observations should be made in a dark room, and the width of the slit giving optimum results should be chosen empirically. The light should be fixed in position relative to the slit.

The quinine obtained from the blood is dissolved in 10 c.cm. of saturated ammonium sulphate solution, and 5 c.cm. of the solution transferred to another of the gauged test tubes. Into five other test tubes of the series are introduced 5 c.cm. of various standard solutions of quinine in saturated solution of ammonium sulphate of strengths increasing from 1 mg. in 500 c.cm. to 1 in 100 c.cm. To each of the six test tubes is then added 0.05 c.cm. of Tanret's reagent and thoroughly mixed at once by shaking. After a quarter to half an hour the tube is "matched" with the standards in the nephelometer, and thereby an approximate idea of the strength of the quinine is acquired. If this is less than 1 mg. in 500 c.cm. the turbidity will be too slight for any but very rough estimation. If more than 1 mg. in 100 c.cm., dilute the remaining 5 c.cm. of quinine solution with 10 c.cm. of saturated ammonium sulphate solution, take 5 c.cm. of the diluted solution and add Tanret's reagent, mix thoroughly, and match with a series of fresh standards. The dilutions at which we find our judgement of turbidity most accurate, using 13 mm. test tubes, lie between 1 mg. in 200 and 1 in 300 c.cm. The final matchings are made with a series of standard quinine solutions of which the strengths decrease from 1 mg. in 200 c.cm. to 1 in 300 c.cm.

Results Obtained with Pure Quinine.

The alkaloid was given dissolved in a saturated watery solution of ammonium sulphate; 5 c.cm. of the solution was taken for each estimation.

Given Milligrams in 5 c.cm.	Found Milligrams in 5 c.cm.	Percentage Error.
0.0200	0.0192	- 3.9
0.0222	0.0217	- 2.2
0.0233	0.0227	- 2.5
0.0222	0.0217	- 2.2
0.0192	0.0189	- 1.7
0.0200	0.0200	Nil
0.0200	0.0202	+ 1.0
0.0250	0.0254	+ 4.2

The observer did not know the correct results beforehand. Those shown above were obtained in a preliminary trial with test tubes gauged only externally.

We have no doubt they can be improved on, and also that, with suitable flat-bottomed tubes 2.5 c.cm. of solution would suffice, in which case it would be possible to estimate 2.5 mg. of quinine with the same degree of accuracy.

Results with Defibrinated Sheep's Blood to which Known Amounts of Quinine Solution had been added.

Five cubic centimetres of blood were used for each estimation.

Milligrams of Quinine given in 5 c.cm. of Blood.	Milligrams Found.	Percentage Error.
0.0303	0.0312	+ 3.1
0.0200	0.0192	- 3.8
0.0200	0.0196	- 2.0
0.0178	0.0172	- 3.5

The amounts shown are such as do actually occur in the blood of patients taking large doses of quinine.

Nephelometric Estimation of Quinine in Urine.

To every 100 c.cm. of urine add 5 c.cm. of 25 per cent. solution of neutral acetate of lead, containing also 2½ per cent. of acetic acid. Mix and then add 5 c.cm. of saturated watery solution of ammonium sulphate. Filter until a clear filtrate is obtained. Test it for absence of lead by receiving a drop on filter paper moistened with a sulphide solution. If lead is found (very exceptional), the above

defaecation process must be omitted, although troublesome emulsions with ether may then be expected. Reckon 100 c.cm. of filtrate as representing 92.3 c.cm. of the original urine.

Test the filtrate with Tanret's reagent; if it gives any turbidity, dilute it with known volumes of water until it just ceases to do so. This is necessary because the 10 c.cm. of saturated ammonium sulphate solution added to the quinine extracted will dissolve only about 1 mg.

Take 10 c.cm. of this "defaecated" (and, if necessary, diluted) urine in a small stoppered cylinder, and filter; dissolve in it 5 grams of ammonium sulphate. Extract with three successive 7 c.cm. lots of purified ether, and so get rid of any "oily matter." Alkalize it with strong ammonia solution, and extract the quinine with three successive lots of ether, transferring each lot as removed to one of the gauged test tubes. Then proceed as already described for blood estimation.

Estimation of Quinine in Urine.

Milligrams of Quinine given in 10 c.cm. Urine.	Milligrams of Quinine found in 10 c.cm. Urine.	Percentage Error.
0.1000	0.0995	- 0.5
0.058	0.0552	- 6.1
0.0833	0.0832	- 0.2
0.1205	0.1232	+ 2.2
0.0400	0.0401	+ 0.2

Tanret's Reagent.

Dissolve 1.35 grams of mercuric chloride in 70 c.cm. of water, and 5 grams of potassium iodide in 20 c.cm. of water in a 100 c.cm. measuring flask. Pour the mercuric solution into the iodide solution under agitation and fill up with water to the mark.

Purification of Ether.

One litre of methylated ether is shaken up thoroughly for five minutes with four successive 100 c.cm. lots of saturated aqueous solution of sodium bisulphite in a separating funnel, allowing half an hour for each separation before running out the watery solution. Wash with 50 c.cm. of half-saturated sodium chloride solution. The ether is then shaken with 50 c.cm. of water plus a little phenolphthalein and enough sodium hydrate solution to give a permanent alkaline reaction. The separated ether is further purified by distillation. It must give no reactions for aldehydes or ketones, as otherwise a Tanret turbidity with the residue of an ether extract would be inconclusive, and nephelometric estimations of quinine would be inaccurate.

As a supremely delicate test for ketones the mercuric cyanide reagent of Scott Wilson can be strongly recommended. As a test for aldehyde (probably much less sensitive) the well-known Schiff's sulphurous acid fuchsin solution may be used.

The ultimate criterion should be that no turbidity develops when the ether is shaken with excess of the Scott Wilson reagent.

Scott Wilson Reagent.

Mercuric cyanide	...	...	0.5 gram
Sodium hydrate	...	...	9.0 grams
Water	...	...	60 c.cm.
0.7268 per cent. silver nitrate solution	...	...	20 c.cm.

The mercuric cyanide and NaOH is dissolved in water, and the silver solution is run in under constant stirring.

THE American National Board for Vocational Education estimates that 100,000 of every 1,000,000 soldiers sent to Europe will return during the first years of the fighting, and that 20,000 of these will need some kind of vocational re-education. A comprehensive Federal scheme for training and placement in wage-earning occupations has been drawn up which includes a central administrative agency in Washington with similar public, semi-public, and private agencies, and the establishment of "curative workshops for the treatment of war cripples, together with a complete system providing for subsistence and pay during the period of re-education."