Special Techniques

The Reaction with Artificial Bilirubin

A small vessel is filled with a freshly made solution of azo-bilirubin. To this end 5 mg. bilirubin is dissolved in 100 c.cm. chloroform; 10 c.cm. of this solution is evaporated under CO_2 in a water-bath until it is nearly dry. About 80 c.cm. of a fluid consisting of 50 per cent. alcohol which contains 10 c.cm. of the above-mentioned buffer solution of pH 6.6 to every 100 c.cm. is added to the residue. The buffer alcohol is heated for some time in order to evaporate the chloroform. Next, the fluid is brought to room temperature, and to it is added 20 c.cm. of the reagent, consisting of (A) 1 gram sulphanilic acid, 15 c.cm. of 25 per cent. HCl, with distilled water up to 1 litre; and (B) 0.5 per cent. sodium nitrite in aqueous solution.

Ten c.cm. of Solution A is mixed with 0.3 c.cm. of Solution B shortly before use. The buffer containing alcohol is added to the fluid after the reagent, and the total quantity is brought up to 100 c.cm. The pigment solution which is to be examined must be left in the dark for fifteen minutes in order to complete the "coupling." Several solutions, all differing by 5 per cent., are made from the azo-bilirubin which has thus been obtained. In this way the weakest solution has half the strength of the original solution, and thus equals 1 in 400,000 bilirubin. One of the vessels is now filled in turn with these different solutions, and the height is determined to which it must be raised in order to obtain a similar light intensity to the other half of the field of vision. The readings of the numbers are marked on the ordinates of a millimeter paper scale and the corresponding units of bilirubin are marked on the abscissae. This constitutes the standard curve of the instrument.

The Direct Reaction

Two c.cm. reagent is added to 1 c.cm. serum. The solution is left in the dark for five minutes, 2 c.cm. of water is added, and then the alcoholic buffer solution up to 10 c.cm. The "coupling" is complete after fifteen minutes. This method keeps the solution perfectly clear for several hours, and a slight turbidity appears only after twenty-four hours. If the serum contains a great amount of bilirubin, and if the azo-solution has, in consequence, a very bright colour, then it must be diluted as often as necessary with 25 per cent. buffer alcohol.

The Indirect Reaction

Two c.cm. serum is mixed with 4 c.cm. alcohol (96 per cent.) and centrifugalized. The clear supernatant fluid is placed in a test tube. Four c.cm. of this solution is placed in a second test tube, and 0.5 c.cm. reagent and 1 c.cm. alcohol are added to it. Wait one or two minutes to obtain maximal colour before using the colorimeter. The dilution factor is 4.

$$\frac{20}{7} \times \frac{5.5}{4} = \frac{5 \times 5.5}{7} = \frac{27.5}{7} = 4 \text{ approx.}$$

It has not been possible to improve this reaction, apart from also employing the filtered (green) light for quantitative determination. We do not commit a grave error by adhering to our original technique in this case, as the quantity of pigment adsorbed to the albuminous precipitate is small in these circumstances.

In these investigations we initially used different proprietary preparations for making the necessary bilirubin solutions. These, however, did not all give, in solutions of the same strength, a quantitatively similar light absorption after diazotizing. We have therefore standardized our instrument with chemically pure bilirubin, with which Professor H. Fischer (Munich) has kindly provided us. An examination of different preparations with the standardized instrument showed that the bilirubin preparations of Hoffman La Roche (Basel) and Fraenkel Landau (Berlin) are exactly similar to Professor H. Fischer's pure preparation.

Summary

This paper describes an improved technique for the determination of bilirubin in the serum by means of the diazo method.

The first improvement is the colorimetric determination in monochromatic light by means of a dimming wire

gauze, instead of the fluid for comparison which was formerly used. This instrument is standardized with azo-bilirubin, derived from chemically pure bilirubin.

The second improvement is the prevention of the adsorption of bilirubin to the albuminous precipitate which occurred when the old technique was followed. This result is achieved by adding, in suitable proportions to the serum, a mixture of reagent, diluted alcohol, and a buffer.

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THE ANTE-NATAL USE OF QUININE

BY

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Several references to the advantage of giving quinine in small doses in the last few weeks of pregnancy have appeared from time to time in the journals. Mitchell¹ (1930) was enthusiastic, and Gair Johnston, Hewetson, and Green-Armytage wrote letters to the *British Medical Journal* on the subject in August, 1933. Special attention was called to the easy labour of the malarial patient under treatment with quinine.

This paper deals with a series of one hundred cases—sixty-six primiparae and thirty-four multiparae—who have been given quinine hydrochloride during the last weeks of gestation. For comparison, a series of the same number of primiparae and multiparae has been taken; in each series toxæmias in any form, and gross pelvic contraction, have been excluded, but otherwise the cases have been selected from the anté-natal booked patients of Queen Charlotte's Hospital.

Pharmacology

Quinine has long been credited with oxytocic properties. It has a direct action on involuntary muscle; in weak concentration it stimulates contraction, but in strong concentration it depresses (Sollman)²; its action is most marked on the uterine muscle, but in animals the intestinal muscle is also effected and splenic contraction also occurs. In the human uterus in labour, however, Bourne and Burn³ showed it had very little effect in hastening delivery, for while it increased the frequency of contractions they were not as powerful. Numerous workers have agreed that it is of no value in the induction of labour except in fully mature or post-mature cases, and medical inductions with quinine and pituitrin have given the same percentage of success when the quinine has been omitted. Quinine has also general tonic properties. It is a general protoplasmic stimulant in small doses, but this is only a transitory augmentation of activity, and in larger doses it is a protoplasmic poison (Cushny).⁴ Its tonic and stomachic action is well known, while its antipyretic properties and specific action in malaria need no mention here. With regard to its increasing resistance, it causes first a mild leucocytosis (followed soon afterwards by a leucopenia) and in higher concentrations it is detrimental to phagocytosis and agglutination (Cushny).⁴

The drug is excreted by the kidneys, but the greater proportion absorbed is destroyed in the body (60-70 per cent.). It appears in the urine fifteen to twenty minutes after ingestion, and the greatest concentration independent of the dosage is present in six to ten hours, though small quantities may be found in the urine for twenty-four to forty-eight hours. It reaches the foetus through the placenta, and has been recovered from foetal urine, liquor amnii, and placental tissue (Dilling and Gemmell).⁵ Excretion by the foetus is thought to be slow, and concentrations of 1 in 100,000 are likely to cause intrauterine death.

Small doses cause marked disturbance in some people (quininism), usually in the nature of disturbance of hearing

and sight, mental confusion, and depression. Skin eruption is perhaps the commonest manifestation of idiosyncrasy, and a large variety of scarlatinal and urticarial rashes have been described (Sollman and Cushny).

Torland described a severe rash associated with foetal death. A possible test for idiosyncrasy is the application of a strong solution of quinine to the scarified skin. In one of the cases given quinine a severe erythematous rash developed after three doses of 5 grains (taken each night) and premature labour followed, but not foetal death (Case 1). One baby developed a miliary rash after twenty-four hours, which may have been caused by quinine. In two patients also the quinine had to be discontinued on account of sickness and headache, in one after six and in another fourteen days. Three cases felt sick for the first three doses, but then continued without further trouble. In this series there was therefore a 3 per cent. idiosyncrasy to the drug.

Dosage and Duration of Medication

The aim in this series was to commence quinine at about the thirty-sixth week of gestation, and to continue until the onset of labour. Quinine hydrochloride in 5-grain tablets was given at night in each case. Labour commenced at varying times—after three days in one case and after fifty-four days in the longest of the series.

The following table shows approximately the number of doses of quinine given.

Onset of Labour						
Under 7 Days	7-14 Days	14-21 Days	21-28 Days	28-35 Days	35-42 Days	42-56 Days
4	12	18	23	17	18	8

There appeared to be no relation between the number of doses and the duration of labour. With regard to the period of gestation at which labour commenced fewer cases of the quinine series went past the fortieth week of gestation calculated in the usual method from the last menstrual period.

The following table shows a comparison of the quinine and control series.

Week at which Labour Commenced		36th	37th	38th	39th	40th	41st	42nd	43rd
Quinine	Primiparae ...	1	4	8	15	29	7	1	1
	Multiparae ...	2	1	8	4	15	2	1	1
Control	Primiparae ...	—	3	8	10	24	11	8	2
	Multiparae ...	1	2	4	6	13	3	3	2

Ante-natal Results

Except for the five cases already mentioned as suffering through idiosyncrasy, all cases felt and looked well. No nausea was experienced with the tablets. In quite a number muscle tone was so good that abdominal palpation was difficult, and in a small proportion the uterus appeared more irritable than normal. The incidence of premature labour was not very different from that in the control series, but in Case 1 it seemed responsible for the onset of labour.

Foetal and Neo-natal Morbidity

One stillbirth and two infant deaths occurred in the quinine series. In the stillbirth the case was one of prolonged labour, with inertia, slow dilatation, and the early passage of meconium. The foetal heart was not heard after sixty hours' labour; I do not think quinine can be held responsible. The infant deaths were due in one case to marasmus (on the fourteenth day, a full-term child, weighing 5 lb. 7 oz. at birth, which steadily lost weight),

and in the other following an operation for sacrococcygeal tumour. The control series contained three stillbirths from prolonged labours, and one infant death from bronchopneumonia.

Dilling and Gemmell, in their investigations on quinine for induction, found a high incidence of the early passage of meconium (34.8 per cent. compared with 8 per cent. in normal cases). This was not noticed in the present series, and there was no difference in the progress of the children after birth. While King and Dilling and Gemmell have each considered the higher concentrations injurious to the foetus, although not necessarily increasing the still-birth rate, I do not think in the smaller dosage continued over several weeks there is any evidence to suggest foetal distress, even where idiosyncrasy has been observed. The smallest child in the series weighed 5 lb. 5 oz. and the largest was 9 lb. 6 oz., with an average for all cases of 7 lb. 1 oz.

Uterine Contraction and Duration of Labour

Clinically, where quinine had been given the contractions appeared to be frequent and of good quality, and progress was good in most cases; the labour ward sisters considered the cases had benefited from the medication. On estimating the hours of labour in comparison with the control series, however, the results were disappointing, and very little, if any, benefit was observed, while in addition several bad cases of inertia occurred in the quinine series.

The time of onset of labour in the following tables has been estimated from when the patient first felt painful contractions of any sort, and not, as in Mitchell's series, from "when the patient has to stand still and hold a chair with each pain."

The second stage, while difficult to estimate accurately, is calculated from the routine figures on the labour ward sheets in each case.

Total Duration of Labour

	Primiparae		Multiparae	
	Quinine	Control	Quinine	Control
Under 5 hours ...	1	2	14	14
5 to 10 hours ...	11	8	12	12
10 to 18 hours ...	25	26	5	5
18 to 24 hours ...	5	9	1	2
24 to 35 hours ...	11	9	1	1
36 to 48 hours ...	1	4	—	—
48 hours and over ..	9	8	1	—

Duration of Second Stage

	Primiparae		Multiparae	
	Quinine	Control	Quinine	Control
Under 1 hour ...	28	32	33	32
1 to 1½ hours ...	10	15	1	1
1½ to 2 hours ...	12	5	—	1
2 hours and over ...	13	14	—	—

In both series of cases twenty-one primiparae had labours exceeding twenty-four hours. In the quinine series six of these cases were typical examples of primary inertia, with labours extending over forty-eight hours. The longest case was 101 hours for the first stage—an occipito-posterior presentation with pains of poor quality (Case 2); four others were occipito-posterior, while one

of the six was occipito-anterior with pains of just poor quality. Four of these inertias were delivered with forceps. The remainder of the labours exceeding twenty-four hours included one extended breech and twelve occipito-posterior presentations. In the multiparae one case which had inertia with her first baby had a labour of fifty-five hours, with weak, ineffective pains (Case 3).

Forceps Incidence, Caesarean Section, and Post-partum Loss, etc.

Eight cases in the quinine series had forceps deliveries, and of these, seven were occipito-posteriors needing manual rotation. In the control series two cases of occipito-posterior were delivered normally after manual rotation, and eight cases were delivered by forceps. Caesarean section was performed in three instances for disproportion. Two of these cases were interesting, in that drug induction with pituitrin was first tried and repeated in twenty-four hours at the fortieth week of gestation with the idea of a trial labour (Cases 4 and 5). The uterine muscle was apparently not more responsive to pituitrin after the quinine medication.

No excessive loss occurred post partum in either series of cases, but the numbers are too small to be of any significance. One multipara, however, who had had a retained placenta with her previous pregnancy, had a perfectly normal third stage. Retraction of the uterus was also satisfactory. Mitchell thought this an important factor in reduction of sepsis, but to consider quinine responsible for this is not justifiable, as retraction was quite satisfactory in the control series. Infection in these cases was never of any serious consequence; no haemolytic streptococci were cultured in any case. One multipara who had had quinine, however, died of pulmonary embolism on the thirtieth day. Femoral thrombosis was present, but no evidence of infection or laceration of the genital tract was seen at any stage (Case 6).

Illustrative Case Records

Case 1.—A. S., aged 36 years, a second multipara, was given quinine at the thirty-sixth week. After the second dose (5 grains each night) she had headache and nausea, and after the third dose had by morning developed an extensive erythematous rash, which was a little itchy, and mild ear and eye symptoms. By evening labour had commenced, and she was admitted to hospital with the head on the perineum after two hours of pains. Normal delivery of a living child (5 lb. 13 oz.) followed. Mother and child progressed satisfactorily, the rash disappearing after three days.

Case 2.—A. W., aged 25 years, a primipara, had quinine for forty-six days previous to labour, which did not commence until three weeks after the estimated date. She was an occipito-posterior presentation, with poor, ineffective pains, and early rupture of membranes. Dilatation of the cervix was slow, and after 101 hours an anterior lip could be pushed up; manual rotation was performed and delivery with forceps. The child's condition was satisfactory, and it weighed 7 lb. 14 oz. This case developed an acute sacro-iliac subluxation, which was treated by manipulation and plaster, with good result.

Case 3.—M. N., aged 25 years, a second multipara, had quinine for fourteen days previous to labour. Weak, ineffective pains and backache were present for forty-eight hours, but then improved, and further progress was good. The labour lasted fifty-five hours in all, and the child weighed 5 lb. 10 oz.

Case 4.—N. D., aged 21, a primipara, had quinine for forty-three days before Caesarean section for disproportion. At the fortieth week of gestation (after thirty-four doses of quinine), medical induction with oil bath enema and six doses of two units of pituitrin at hourly intervals was tried, and repeated after twenty-four hours without any result. The convalescence after the operation was normal, and the child weighed 8 lb.

Case 5.—E. P., aged 23, a primipara, was similar to Case 4. Quinine was given for twenty-eight days, and then medical induction was tried and repeated without result. The child weighed 9 lb. 6 oz.

Case 6.—D. H., aged 28, a second multipara, had quinine for twelve days before labour commenced. She was admitted to hospital with the head on the perineum, and normal delivery followed. The total duration of labour was one hour thirty-five minutes, and the child's weight 7 lb. On the evening of the third day she had a small pulmonary embolism; this recurred twice in the next two weeks, and on the thirtieth day she had a large embolism, causing death. At necropsy there was no evidence of infection or laceration in the genital tract; the left femoral vein was thrombosed, and several emboli were present in the lungs.

Conclusions

Quinine, given in small doses in the last weeks of gestation, acts as a general tonic and stimulant, and the patients feel well and are often improved. Idiosyncrasy is likely in a small percentage of cases, and its manifestations may include skin reaction and the onset of premature labour. There is no evidence to suggest any foetal toxicity or increase in foetal mortality, and, apart from idiosyncrasy, little risk of premature labour. The effect of the drug on the duration of labour is of doubtful value. Clinically, the pains appeared to be improved, but in comparison with the control series no significant difference was evident. Inertia is certainly not eliminated. The general tonic effect may have some beneficial results in reducing the liability to infection through increased resistance.

My thanks are due to the Queen Charlotte's Hospital honorary medical staff for their assistance and advice, and to the Sisters of the hospital for their aid in compiling these results.

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- ⁵ Dilling and Gemmell: *Journ. Obstet. and Gynaecol. British Empire*, 1929, xxxvi, 352; 1930, xxxvii, 529.
- ⁶ Torland: *Journ. Amer. Med. Assoc.*, 1928, xc, 1190.
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Further information has now been received about the post-graduate course and tour in Switzerland from August 15th to 23rd, previous notification of which appeared in the *Journal* of April 21st. The Basel Medical Association will entertain members of the course on the evening of August 15th, and on the following morning there will be a lecture at the Basel municipal hospital and a visit to the new maternity hospital. The same evening there will be an official dinner at Zürich, with lectures on the new hospital buildings there. The morning of August 17th will be devoted to a visit to the Neumünster hospital, the psychiatric clinic, and the children's hospital. In the evening, at Zürich, there will be an informal welcome by the medical associations of Lucerne, and the following morning will be spent in study of the reconstructed and enlarged cantonal hospital and two local clinics. The official post-graduate course will open at Bern on the morning of August 19th with lectures on the general aspects of hospital treatment, and further lectures on cognate subjects will follow at the University on the three next mornings, with discussions. The formal course will conclude at Leysin on August 23rd, and the five following days will be spent in visits to Arosa, Davos, and St. Moritz. The whole course is arranged in three sections—namely, Basel and Lucerne, Bern and Leysin, and the Canton Grisons tour, the respective fees being 90, 150, and 150 Swiss francs. Tickets should be booked before July 1st. Further details are obtainable from the secretary of the post-graduate course, Obergundstrasse 13, Lucerne.