Anticoagulants such as phenindione, while having no effect on graft patency, may have a distinctly beneficial influence on the survival of the limb should the graft thrombose. The effects of sympathectomy appear uncertain, but if there is any beneficial effect it is on limb survival after graft occlusion. Sympathectomy does not favourably influence graft patency.

There is a continued striking improvement in the clinical state of many of the legs even when grafts have occluded, as shown by the disappearance of previously palpable pedal pulses. That is to say, limbs that are technical failures remain therapeutic successes. The reason for this is not clear, but to some extent the improvement may be due to increased perfusion of the deep femoral artery following the clearing of the origin of this vessel prior to implanting the upper end of the graft. Despite these apparently disappointing results it must be stressed that they do represent salvage surgery. Forty-one of the patients had rest-pain, ulceration, and impending or frank gangrene; amputation was therefore the only practical alternative treatment in most of them. In fact, only 15 limbs were subsequently amputated, this giving an overall salvage rate of 65%. The majority of these latter patients either have a limb with palpable pedal pulses or one showing marked symptomatic improvement over its pre-operative state. Viewed in this light we believe that the surgical treatment of this increasingly common affliction well merits further endeavour.

Summary

The late results of homografts and prosthetic grafts in the management of femoro-popliteal occlusion are compared.

Of 23 homografts followed for periods up to eight years 12 are still functioning. Four of the latter became aneurysmal and one has been replaced and remains functional.

Of 24 teflon tubes inserted, 22 failed within a period of 20 months; two are still functioning.

The effects of phenindione and sympathectomy on graft patency and limb survival are discussed.

The "salvage" rate of limbs otherwise doomed to amputation was 65%.

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REFERENCES

- Ashton, F., and Rains, A. J. H. (1960). *Lancet*, **1**, 19.
 Bellman, S., and Löfström, B. (1961). *Acta chir. scand.*, **121**, 384.
 Crawford, E. S., De Bakey, M. E., Morris, G. C., and Garrett, E. (1960). *Surgerv.*, **47**, 79.
 De Bakey, M. E., Crawford, E. S., Cooley, D. A., and Morris, G. C. (1958). *Ann. Surg.*, **148**, 306.
 Edwards, W. S. (1960). *Surg. Gynec. Obstet.*, **110**, 714.
 Laufman, H. (1960). *Surg. Clin. N. Amer.*, **40**, 153.
 Warren, R., John, H. T., Shepherd, R. C., and Villavicencio, J. L. (1961). *Surgery*, **49**, 1.
 Whitman, E. J., Jones, J. M., and Ivins, J. C. (1961). *Arch. Surg.*, **82**, 153.

ANTENATAL PREDICTION OF HAEMOLYTIC DISEASE OF NEWBORN

COMPARISON OF LIQUOR AMNII AND SEROLOGICAL STUDIES

BY

A. H. C. WALKER, M.B., B.S., F.R.C.O.G.

Reader, Department of Obstetrics and Gynaecology,
University of Manchester

AND

R. F. JENNISON, M.D., M.R.C.P.

Clinical Pathologist, St. Mary's Hospitals, Manchester

When faced with a pregnant, sensitized, rhesus-negative woman it would be advantageous to have a reliable guide to whether the maternal and foetal blood groups were in fact incompatible in this pregnancy, and if this were so an indication of the severity of the haemolytic process would assist in the management of the case. A knowledge of the past history alone is not always enough, because the baby may be rhesus-negative if the father is heterozygous, and even with a homozygous father the severity of the disease may vary from pregnancy to pregnancy.

As a criterion for assessing the severity of haemolytic disease the maternal antibody titre has been shown by some workers (Kelsall and Vos, 1952; Allen and Diamond, 1958; Tovey and Valaes, 1959; Dique and Wrench, 1959) to be correlated with the severity of the haemolytic process.

Another approach to the problem is the study of liquor amnii. Bevis (1956) showed that spectrophotometric analysis of the liquor in cases of haemolytic disease revealed an increase in blood pigments. Continuing this line of investigation, Walker (1957) has shown in a preliminary report that it is possible to predict with some degree of accuracy whether the baby will be affected or not by a study of the shape of the spectral absorption curve relative to bilirubin. The presence of oxyhaemoglobin is not important because it is only a reflection of blood in the liquor. More recently M. Mayer (personal communication, 1960), Cary (1960), and Liley (1961) have reported similar success by the use of this method.

If bilirubin could be measured more accurately in the liquor it might be possible to make a more objective prediction. It was decided, therefore, to examine the liquor in a series of cases for the presence of bilirubin, both qualitatively and quantitatively, and, in view of the results obtained by other workers, to carry out antibody titres simultaneously. From October, 1959, to October, 1961, 156 patients have been so examined; all the predictions were made on the analysis of the spectrophotometric curve. The quantitative bilirubin levels and the antibody titres were analysed retrospectively.

Material and Methods

Liquor Amnii Specimens

The technique of obtaining liquor amnii requires to be stressed, for the procedure is not a difficult one if the actual site for paracentesis is accurately determined. It will be appreciated that the foetus *in utero* is in an attitude of flexion, and with the arms and legs flexed there is a definite gap between the upper and lower

limbs, and it is at this point that there is the pool of liquor amnii. On palpation this gap can easily be located because of a lack of resistance. It is possible, therefore, over quite a definite small area to dimple the skin, and it is in this area that the needle should be inserted (Figs. 1, 2, and 3). The site is always on the side opposite to the baby's back and usually below the umbilicus, but is at a higher level in breech presentation and with occipito-posterior position. Occasionally difficulty may be encountered in obese patients and when the placenta is on the anterior uterine wall. However, liquor can be obtained successfully in 95% of cases, and in only 5% of the successful taps was a blood-stained specimen obtained.

A lumbar puncture needle is inserted under local skin anaesthesia. To obtain a specimen of liquor amnii the

FIG. 1.—Paracentesis is performed at point marked X, which is always on side opposite to baby's back and usually below umbilicus.

FIG. 2.—A metal disk has been placed on umbilicus, and a smaller one at site of paracentesis. These confirm that the point for paracentesis is on side opposite to baby's back, and is between the flexed arms and legs.

needle should not be of too fine a bore because it may become blocked with vernix caseosa.

No patients have complained of any discomfort during or after the procedure and there have been no complications in at least 500 cases in the past six years.

Liquor amnii was obtained by this method from the 156 mothers with anti-D or C+D antibodies. (Liquor was also obtained from 17 other mothers who were rhesus-negative and had other antibodies discovered at routine testing; these formed the control group.) Most of the specimens were obtained between the 32nd and 34th weeks of pregnancy because it has been shown that tests done later than this give false predictions (Walker, 1957) as a result of the gradual disappearance of bilirubin in the last weeks. This has since been confirmed (Wild, 1961). All the predictions were made by one of us (A. H. C. W.) and were classified as (1) unaffected; (2) serologically affected only—that is, Coombs-positive but not requiring treatment; (3) mildly affected; (4) moderately affected; and (5) severely affected. The last three grades indicated the likelihood of exchange transfusion being required more or less urgently and the possibility of multiple transfusions being required. Severely affected predictions frequently denoted that the foetus might succumb prior to, during, or shortly after labour.

Blood Specimens.—Maternal venous blood was obtained at the same time as the liquor and, on occasion, before and after the paracentesis. Cord blood was obtained at delivery except in the case of most stillbirths.

Spectrophotometry.—Direct examination of liquor amnii was carried out in a Unicam S.P. 500 spectrophotometer after the liquor had been centrifuged and the supernatant fluid filtered. An absorption curve was plotted between the wave-lengths 350 and 700 m μ on semilogarithmic paper and examined for an absorption peak due to bilirubin.

Bilirubin Estimations.—These were carried out after the method of Powell (1944) adapted for use in a Unicam S.P. 500 spectrophotometer. A standard curve was prepared from pure bilirubin (B.D.H.) over the range 0–2 mg./100 ml., and 1 ml. of liquor, 0.2 ml. of diazo reagent, and 2.8 ml. of sodium benzoate-urea were used. (The low protein content of the liquor enables increased amounts of it to be used without protein precipitation.)

FIG. 3.—Lateral radiograph, showing that the paracentesis needle is inserted between the flexed arms and legs of foetus.

Antibody Titration.—Anti-Rh antibodies (anti-D or anti-C+D), previously shown to have been present, were titrated by means of the indirect antiglobulin technique. Twofold serial dilutions of two drops of maternal serum were made, normal saline solution being used as the diluting medium. To each tube were added two drops of an 8% suspension of fresh washed group O Rh-positive cells, type R₁r, the tubes capped and incubated for two hours at 37° C. The supernatant fluid was then removed and the cells were washed three times in normal saline. To one drop of an approximately 4% suspension of these cells on a slide was added one drop of a standard anti-human-globulin serum. That dilution giving agglutination just visible to the naked eye after six minutes' gentle rocking was taken as the end-point of titration. In order to minimize personal errors, all tests were carried out by one person only over a long period. Appropriate controls were used in each batch of titrations.

Results

Analysis of Absorption Curve

On the 156 cases studied in this way, 142 (91%) correct predictions were made of the degree of affection of the infant at birth. This overall improvement, compared with that of 75.2% in the preliminary report (Walker, 1957), was due to the fact that a higher proportion of cases, 87.1% as against 60.4%, were tested before the 35th week. Table I shows that 136 cases

TABLE I

	Cases	Correct Forecasts
Tested before the 35th week ..	136	126 (92.6%)
„ after „ 35th „ ..	20	16 (80%)

were tested before the 35th week, with 126 correct forecasts (92.6%). After the 35th week the accuracy was only 80%, although this is an improvement on that of 50% in the preliminary report, at which time the cases were being seen nearer to term.

Whereas in our original communication we did not always have information concerning the husband's genotype, in this series we obtained better co-operation, although frequently not until after the prediction had been made. Our present results can therefore be further studied according to the husband's genotype. In 84 cases the husband was homozygous and in 72 heterozygous.

In the group where the husband was homozygous there were 81 correct forecasts (an accuracy rate of 96.4%). Further, it will be recalled that bilirubin tends to disappear from the liquor amnii after the 35th week. Two of the incorrect forecasts in this group were in cases tested after the 35th week. Table II shows that the accuracy rate in the homozygous group was 98.6% when the test was performed before the 35th week. In the two late cases where the forecasts were incorrect the cord blood was Coombs-positive. The forecasts had suggested unaffected babies. These results illustrate the disappearance of bilirubin in late tests, even though the

TABLE II.—Homozygous Father

	Cases	Correct Forecasts
Tested before the 35th week ..	72	71 (98.6%)
„ after „ 35th „ ..	12	10 (83.3%)
Total	84	81 (96.4%)

antibody titre in one case was 1,024. Seven babies were Coombs-negative and were predicted correctly. These results suggest extramarital pregnancy or faulty genotyping.

In the group where the husband was heterozygous (Table III) there were 61 correct forecasts, an accuracy rate of 84.7%. Further analysis according to whether the test was performed before or after the 35th week again shows the value of an early test, and it should

TABLE III.—Heterozygous Father

	Cases	Correct Forecasts
Tested before the 35th week ..	64	55 (85.9%)
„ after „ 35th „ ..	8	6 (75%)
Total	72	61 (84.7%)

be noted that in this group 48 babies were Coombs-positive and all but three were predicted. Of the remaining 24 babies who were Coombs-negative 16 were predicted as negative. The other eight were incorrectly forecast as likely to be mildly affected.

Liquor Bilirubin Levels

The distribution of bilirubin levels in liquor amnii obtained from three groups of patients is shown in Table IV. Because of the ethical problem of doing paracentesis on normal women the estimations in group 1 were done on liquor from women with antibodies not known to cause haemolytic disease *in utero*—for

TABLE IV.—Distribution of Bilirubin Levels in Liquor Amnii

Bilirubin (mg./100 ml.)	Group 1 (Normal)	Group 2 (Coombs-negative)	Group 3 (Coombs-positive)
0-0.09	11 (3)	18 (4)	32 (12)
0.1-0.19	6	8	40 (1)
0.2-0.29	—	3	20 (1)
0.3-0.39	—	1	12
0.4	—	—	19 (1)

Figures in parentheses indicate number of cases in which the paracentesis was done after 35 weeks.

example, anti-P and anti-Lewis. Group 2 comprises the patients with antibodies but whose babies were Coombs-negative and showed no evidence of haemolytic disease at birth or later. Group 3 consists of the patients whose babies were Coombs-positive and had haemolytic disease of varying degree. The bilirubin level in the liquor of patients carrying unaffected babies varied from 0 to 0.31 mg./100 ml., with a mean of 0.09 mg. When haemolytic disease was present the range was from 0-3.36 mg./100 ml., with a mean of 0.25 mg. It was only in severely affected infants that the liquor remained jaundiced until birth. Even including these late specimens, a bilirubin level over 0.2 mg./100 ml. occurred in 40% of the liquors in group 3—that is, Coombs-positive pregnancies—as compared with only 8.5% in the unaffected pregnancies (groups 1 and 2).

The level of bilirubin in the liquor becomes more significant if it is related to the severity of the disease in the baby (as measured by the number of babies dying from haemolytic disease or requiring transfusion). Table V shows this analysis in the patients from whom the liquor was obtained before 35 weeks. It will be seen that when the bilirubin in the liquor was less than 0.2 mg./100 ml. only 33% of the infants required treatment, and when the liquor bilirubin was more than 0.2

mg./100 ml. 83% required treatment. Furthermore, the average number of transfusions per surviving Coombs-positive baby was much greater when the bilirubin in the liquor was above 0.2 mg./100 ml.

TABLE V.—Liquor Bilirubin Level Before 35 Weeks Related to Outcome of Pregnancy

Bilirubin (mg./100 ml.)	Total No. of Infants	Coombs-negative	Coombs-positive	Coombs-positive			Percentage		Trans. Index*
				Died from Haemolytic Disease	Exchange Transfused	Top-up Only	Coombs-positive	Dying or Treated	
0-0.09	34	14	20	2	6	1	58	26	0.75
0.1-0.19	47	8	39	4	13	1	83	38	
0.2-0.29	22	3	19	3	14	—	86	77	2.8
0.3-0.39	13	1	12	2	9	—	92	84	
0.4	18	—	18	12	6	—	100	100	

* Number of transfusions per surviving Coombs-positive infant.

Antibody Titres

Titres were estimated in 156 patients at the same time as the liquor was examined, and in 126 the titre was estimated at least once more later.

It was obvious that there was a marked difference in the degree of severity above and below a titre of 32; the results have therefore been analysed with this titre as a dividing-point. It will be seen that, although there is a difference both in incidence and in severity of haemolytic disease above and below this figure in the maternal sera taken before 35 weeks (Table VI), the difference is more marked if the highest titre is considered in the 126 patients whose titre was repeated (Table VII). Of the 126 repeated tests, in only 52 patients was there a rising titre of any degree. Of these, 24 rose from below to above 32, all were Coombs-positive, and 17 required treatment. Four rose but remained below 32, and only one required treatment. The babies of all but two of the patients with rising titres were affected; these two were Coombs-negative, and the rise was twofold and fourfold. The remaining 22 Coombs-negative babies showed no rise in maternal

TABLE VI.—Maternal Antibody Titres at 32 Weeks

Titre	Total No. of Infants	Coombs-negative	Coombs-positive	Coombs-positive			Percentage		Trans. Index
				Died from Haemolytic Disease	Exchange Transfused	Top-up Only	Coombs-positive	Dying or Treated	
4	38	15	23	1	5	—	72	26	0.58
8	13	3	10	—	4	—			
16	17	1	16	2	6	—	87	72	2.28
32	21	2	19	1	10	1			
64	26	2	20	6	11	—			
128	22	2	20	7	11	2			
256	17	1	16	7	9	—			

TABLE VII.—Highest Maternal Titre

Titre	Total No. of Infants	Coombs-negative	Coombs-positive	Coombs-positive			Percentage		Trans. Index
				Died from Haemolytic Disease	Exchange Transfused	Top-up Only	Coombs-positive	Dying or Treated	
4	16	10	6	—	—	—	59	6	0.15
8	8	3	5	—	—	—			
16	8	—	8	—	—	—	88	68	1.99
32	19	2	17	—	9	1			
64	22	5	17	4	9	—			
128	21	5	16	5	9	—			
256	32	5	27	5	19	—			

titre. Fifty-two patients showed no rise in titre but gave birth to Coombs-positive babies; most of these had high titres early in pregnancy, but a few had very low titres, and the only sign of affection was a weakly positive Coombs reaction. In only two instances in which the titre remained below 32 did the babies require treatment; in one of these there was evidence of associated ABO incompatibility.

Discussion

Comparison of the various methods is difficult because of the different mechanisms involved in the tests carried out, but, as would be expected, there is quite a degree of correlation between antibody titres and liquor bilirubin levels. As antibodies are carried over from one pregnancy to another it is obvious that a high antibody level can be associated with a rhesus-negative baby; there are 11 (13%) such cases with a titre greater than 32 (Table VI). Conversely, a normal or low bilirubin in the liquor before 35 weeks may give a false impression of the state of an infant not born until some weeks later if in the meanwhile the titre rises. Of the 27 below 0.2 mg./100 ml. requiring treatment or dying, 19 titres were repeated and 17 showed a significant rise. It is not always possible to forecast whether the baby will be affected or not, as there will be occasional inaccuracies, but if an attempt is made to assess the severity of the disease—that is, whether treatment will be required or not—better forecasts can be made and the tests are obviously of much greater value.

Unfortunately it is difficult to define criteria for the severity of the haemolytic process after the child is born. The cord bilirubin reflects to a certain extent the severity of the disease, but, as is well known, in a hydropic infant the cord bilirubin may be very low. We have attempted to use the number of exchange transfusions required as a measure of the severity. This is also open to criticism because the treatment was not all carried out by the same paediatrician. Also in a number of cases the pregnancies were deliberately terminated early in order to reduce the severity of the disease and these babies had fewer transfusions than if they had been delivered at term. In other words, the forecast given on the prenatal tests at 32 weeks suggests the eventual severity of the haemolytic process if the pregnancy were allowed to proceed to term.

Analysis of the spectrometric curves gives very good results, but it must be remembered that the accuracy is based on a forecast of the degree of affection and the likelihood of exchange transfusions being necessary. Moreover, it is obvious that when low concentrations of bilirubin are present the spectrometric curve reflects more closely the minor changes in pigment level in the liquor than does quantitative measurement by the method used. From comparison of the curves and the estimated amount of bilirubin in individual cases it is evident that the chemical method is sometimes inaccurate, especially at levels below 0.1 mg./100 ml.

Both the curve and the measured bilirubin reflect the degree of the disease in a foetus at the time the paracentesis is performed, and indicate the time that induction should be undertaken. Only 58% of the babies whose liquor before 35 weeks contained less than 0.1 mg./100 ml. of bilirubin were Coombs-positive (Table V), whereas from a knowledge of the distribution of the D antigen in rhesus-positive husbands 70% of the babies would have been expected to be Coombs-positive.

Between 0.1 and 0.2 mg./100 ml. bilirubin, 83% were Coombs-positive, a much greater proportion than genotype distributions would have indicated. Similarly, above 0.2 mg./100 ml. all but four (92%) of the babies were Coombs-positive.

When one considers the number of babies requiring treatment it will be seen that 67% of those with bilirubin less than 0.2 mg./100 ml. did not require treatment. Above 0.2 mg./100 ml. only 7 (13%) of the 35 babies did not require treatment. Three of these untreated babies were delivered early with the object of reducing the severity of the disease. The other four babies (8%) with bilirubin levels in the liquor of more than 0.2 mg./100 ml. were Coombs-negative. The spectral absorption curve also reflected this bilirubin level. The cord bilirubin in each case was higher than the average but within normal limits. There was no evidence of other blood-group incompatibility, and the reason for high liquor bilirubin level has not been explained, although there could be a number of possible reasons unconnected with haemolytic disease. Over 0.35 mg./100 ml. all the babies died or required transfusions.

Except in the pregnancy when antibodies are first discovered, an estimation of the titre can be misleading in that even a very high titre can represent the effect of a previous pregnancy. It is not possible to indicate whether the foetus is rhesus-positive unless there is a rise in titre, but this occurred in only 41% of cases. Of the patients with a titre greater than 32, 12% produced a Coombs-negative infant. Before one can assess the severity of the disease from antibody titre alone it is necessary to know for how long during this pregnancy a particular titre has been present. This relationship between time and titre was well shown by Kelsall *et al.* (1958). It is illustrated also in Tables VI and VII. Ninety-four per cent. of the babies born of mothers with a titre of 64 or more at 32 weeks either died or were transfused if they were Coombs-positive (Table VI); whereas, if the highest titre is considered (Table VII), only 86% required treatment in spite of this high titre, because the titre had not been present for sufficient time.

Broadly speaking, titres at 32 weeks give the same overall forecast as do bilirubin levels at 32 weeks; 87% of the babies with maternal titres of 32 or higher were Coombs-positive and 92% of the liquors with bilirubin more than 0.2 mg./100 ml. were from Coombs-positive babies. But whereas only 72% of the babies with maternal titres of 32 or higher died or required treatment, 87% of the babies with bilirubin levels in the liquor of more than 0.2 mg./100 ml., or the corresponding spectral absorption curve, died or required treatment, showing a more accurate forecasting of the degree of affectedness.

In conclusion it may be stated that if the husband is known for certain to be homozygous and the maternal titre is 32 or rises to 32 or more, the infant will probably require treatment, and the higher the titre the more danger there is to the foetus. But only by examination of the liquor for bilirubin can the state of the individual foetus be assessed and early or late induction decided.

If the liquor bilirubin is below 0.1 mg./100 ml. the infant is unlikely to require treatment and the necessity for treatment increases with the rising bilirubin, so that when levels of 0.4 mg./100 ml. are reached there is a great risk of death. In our experience the lower levels can be more accurately evaluated by the spectrophotometric method than by the quantitative method used in this survey, but at higher levels the two methods correspond more closely.

If the husband is heterozygous the antibody titre is of little value unless a distinct rise in the titre can be detected. In these cases a liquor examination may not only indicate whether the foetus is affected or not but it will also be of great help in deciding when to induce labour if this is indicated.

There are obviously instances when the husband is homozygous, such as first affected babies or patients with particularly bad histories, when a liquor examination would indicate much more exactly what is occurring in the current pregnancy and would therefore enable appropriate measures to be taken in time.

It must be remembered that in a few cases a rise in titre during the last few weeks will result in an affected infant although the liquor analysis was negative.

The possibility that paracentesis may cause a leak from the foetal to the maternal circulation with consequent rise in titre has been raised (Moncrieff *et al.*, 1960). In only 41% of our cases was there a rise in titre (including minimal rises—that is, twofold), whereas Kelsall *et al.* (1959) found a rise in more than 50% of patients in whom no paracentesis was done. Some of this difference may be accounted for by less frequent titre estimations in our series, but, even so, it does not seem likely that amniotic taps were responsible for many rises in titre.

Summary

In a series of 156 rhesus-negative women with antibodies both liquor amnii analyses and antibody titres have been studied as a means of predicting the outcome for the foetus, as judged by the number of transfusions or subsequent death.

By examination of the spectrophotometric curve accurate forecasts of the degree of affectedness were obtained in 91%.

When the bilirubin level measured quantitatively was more than 0.2 mg./100 ml., 87% of the babies required treatment or died.

When the titre was 32 or more, 72% of the babies required treatment or died.

The reasons for discrepancies in forecasts by the various methods are discussed. It is emphasized that it is the examination of the liquor rather than the estimation of the antibody titre which will indicate what is occurring in the current pregnancy.

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REFERENCES

- Allen, F. H., and Diamond, L. K. (1958). *Erythroblastosis Fetalis*. Little, Brown, Boston.
- Bevis, D. C. A. (1956). *J. Obstet. Gynaec. Brit. Emp.*, **63**, 68.
- Cary, W. (1960). *Med. J. Aust.*, **2**, 778.
- Dique, J. C. A., and Wrench, D. L. (1959). *J. Obstet. Gynaec. Brit. Emp.*, **66**, 251.
- Kelsall, G. A., and Vos, G. H. (1952). *Med. J. Aust.*, **1**, 349.
- and Kirk, R. L. (1958). *Brit. med. J.*, **2**, 468.
- (1959). *Med. J. Aust.*, **1**, 488.
- Liley, A. W. (1961). *Amer. J. Obstet. Gynec.*, **82**, 1359.
- Moncrieff, A., Stabler, F., and Norman, A. P. (1960). *J. Obstet. Gynaec. Brit. Emp.*, **67**, 740.
- Powell, W. N. (1944). *Amer. J. clin. Path.*, Tech. Sect., **8**, 55.
- Tovey, G. H., and Valaes, T. (1959). *Lancet*, **2**, 521.
- Walker, A. H. C. (1957). *Brit. med. J.*, **2**, 376.
- Wild, A. E. (1961). *Clin. Sci.*, **21**, 221.