with antimalarial drugs is now carried out almost automatically by most of the European population.

In contrast to this it seems not unlikely that blackwater fever may in the near future occur with increasing frequency amongst the educated African population, especially the children. Antimalarial drugs are increasingly easy to obtain, and skilful advertising in the cinema, press, and shops will certainly make new African converts to malaria chemotherapy. The danger lies in the likelihood that in many cases chemoprophylaxis will not be continuous—either because the desirability of this may not have been appreciated or for financial reasons. If this is so, the African child in a hyperendemic zone will neither be adequately protected from malaria nor be able to build up immunity to it. A similar situation may be produced by the repeated casual treatment of all fevers in African infants with inadequate doses of antimalarial drugs. This may be overstating the case, as the most likely offender —quinine—is being to a certain extent ousted by the synthetic antimalarials mepacrine and proguanil. However, quinine is still sold very widely, and blackwater fever has been described after mepacrine. Blackwater fever after proguanil does not seem to have been recorded. It must be mentioned, however, that proguanil has been in use for only a few years, and then during the period when the importance of the regularity of prophylactic dosage has been very widely accepted and put into practice.

In the paediatric out-patient department in Ibadan only intelligent educated mothers who can afford it are advised to give their children malarial chemoprophylaxis in the form of proguanil. In addition, it is stressed to them that this must be an uninterrupted process and, indeed, should continue throughout life, as otherwise there is a definite danger that the pernicious manifestations of subtertian malaria—that is, cerebral malaria, etc.—may occur in adult life. It is realized that not all authorities would agree with this advice, as they feel that the "normal" process of repeated infection with subtertian malaria that occurs in almost all African children in a hyperendemic zone is inevitable and not such a serious matter as is usually suggested. This has been stressed by the work of Garnham (1949) in the Luo tribe of Kenya and by Swellengrebel (1950) in the African population (Cimarons) of a village in the interior of Surinam. Both authors note that, despite an almost universal infection with *P. falciparum* during infancy and childhood, only a very small mortality, or even morbidity, is attributable to malaria in these areas. It seems unlikely, though admittedly statistically unproved, that similar conditions pertain in Nigeria, where severe and often fatal pernicious attacks of subtertian malaria are seen comparatively frequently in young children and appear to represent the price paid for the development of malarial immunity in the surviving majority.

Summary

The rarity of blackwater fever in Africans, especially in childhood, is discussed. Four cases occurring in Nigerian children are recorded.

The fall in incidence of blackwater fever in the European population of Nigeria is noted.

The possibility of an increase in the number of cases of blackwater fever in the children of educated Africans, following the wider casual use of antimalarial drugs in the home, is considered.

In view of this danger it is advised that only intelligent educated mothers should be advised to give their children chemoprophylaxis—in the form of proguanil. The desirability of continuing this into adult life is mentioned.

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LABORATORY DIAGNOSIS OF URINARY-TRACT INFECTIONS

WITH SPECIAL REFERENCE TO LACTOSE-FERMENTING COLIFORM STRAINS

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The common infecting agents in disease of the urinary tract are organisms of the coliform group. This is a group of immense complexity, and a prolonged bacteriological analysis is necessary before any one member can be accurately described. It is therefore common routine practice to disregard these minor differences and describe them by some all-embracing term such as *Bact. coli* (Ainsworth-Davies, 1950) or simply as "coliform bacilli." It is the purpose of this paper to show that the subdivision of the group into true *Bact. coli* and *Bact. aerogenes* species, and a group containing all other lactose-fermenting Gram-negative bacteria to be called "atypical strains of the coliform group," may be of practical importance. A simple means of achieving this is described, utilizing only information obtainable in 24 hours. The results obtained by this method have been compared with those from one of the standard longer techniques. The data have also been analysed to see if they show any correlation between the nature of the infecting strains and the pathology of the lesions.

The incidence of a variable proportion of *Bact. aerogenes* strains in these infections has many times been reported, and recently attention has also been drawn to the sulphonamide insensitivity and penicillin sensitivity of large numbers of coliform organisms. The fact that nearly all are sensitive to streptomycin and to some of the newer antibiotics (Frank *et al.*, 1950) in no

way detracts from the topical importance of these observations, since it is still routine practice to treat most cases with a sulphonamide on first diagnosis. Furthermore, resistance to streptomycin may rapidly develop, and no new antibiotic is generally available in this country for the treatment of these infections.

Material

Considerable difficulty was experienced in deciding what constituted a urinary-tract infection in the absence of a definite urological diagnosis, and, since clinical diagnosis was frequently made on the result of the laboratory findings, there was in retrospect often little independent evidence.

Numerous authors have shown that contamination occurs in a very high percentage of routinely collected urines, even catheter specimens. There is a considerable probability that even with rigorous precautions as many as 10% of urines may grow organisms the presence of which is not attributable to any discoverable pathological lesion. In a preliminary study of 200 specimens primarily conducted to confirm that there was satisfactory correspondence between wet-film and dry-film diagnosis a large number of contaminated specimens, mostly containing cocci, were in fact noted. Cystoscopic and ureteric specimens were only rarely contaminated, which fact emphasizes the importance of collection technique. Only undoubted cases, therefore, in which there were micro-organisms and a definite excess of pus cells in the centrifuged deposit, and, with one exception, relevant clinical findings, have been accepted as providing infected as opposed to contaminated urines. It is felt that there is need for a critical review of the characters of a urinalysis that constitute grounds for the diagnosis of a urinary-tract infection, and particularly of the significance of albumin.

Cases.—The patients were all the 86 cases with urinary-tract infections in the wards of Guy's Hospital from whom specimens had been sent to the Department of Clinical Pathology for examination, and 51 others in whom the urinary tract was thought to be normal and from whom the contaminating strains studied were derived. Only those patients from whom a lactose-fermenting coliform strain was isolated have been included, but they were otherwise unselected. The specimens were received during the months of November to May; all had been collected by students and nursing staff. Only catheter specimens were accepted for culture from female patients, and from the male patients 18% were catheter specimens and 82% were interrupted mid-stream urines collected with due precautions to avoid contamination.

Strains.—From primary growths on MacConkey plates 165 strains were obtained. When the growth looked pure a representative colony was picked and when more than one organism appeared to be present one of each type was investigated. Of these 165 strains 114 were considered to come from the true cases of infection and 51 to be contaminating urines from the cases that were not clinically infected. Not all contaminants encountered in the latter part of the investigation were studied.

Methods.—All cultural methods were those described by Wilson and Miles (1946). O'Meara's modification of the Voges–Proskauer reaction is found in this department to be more generally useful than Barritt's. All investigations were performed shortly after first isolation.

Penicillin sensitivities were determined in tubes containing 5 ml. of glucose broth with 100 and 20 units per ml. and a control tube. For sulphonamide sensitivities the plate method of Harper and Cawston (1945) with a control plate was employed. The inocula were standardized by the method in use at the Army Vaccine Laboratory (Sayers, 1949; personal communication to J. B. E.), which consists in emulsifying in peptone water the inoculum on a 3-mm. loop, flaming the loop, touching the emulsion with it, and then agitating the loop in a fresh tube of peptone water. This loop is then used for inoculation, and on solid media invariably produces separate colonies. The gas-production test was carefully standardized so that the temperature within the medium—lactose bile salt broth—was 44° C ± 0.5.

Classification of Strains

An attempt was made to apply the Ministry of Health (Report, 1934) classification of coliform strains met with in water in Great Britain.

Table Showing the Number of Strains in Each Type in the Ministry of Health Classification and the Number of Strains in Each Broad Group in Relation to the Lesions from which They Were Isolated

Source of Strains	No. of Cases	No. of Strains	*Bact. coli*		Atypical Coliform Strains						*Bact. aerogenes*	
					Intermediate Strains		Irregular Strains					
			Type I Faecal	Type II	Type I	Type II	Type I	Type II	Other Types Producing Gas at 44° C.	Other Types Not Producing Gas	Type I	Type II
Infected urinary tracts	86	114	57	11	10	6	2	5	6	9	6	2
Normal urinary tracts	51	51	17	4	11	2	4	1	6	1	4	1
Total strains		165	74	15	21	8	6	6	12	10	10	3
			Bact. coli		Atypical Coliform Strains						*Bact. aerogenes*	
Infected urinary tracts			68 (60%)		38 (33%)						8 (7%)	
Normal urinary tracts			21 (41%)		25 (49%)						5 (10%)	
Total strains			89 (54%)		63 (38%)						13 (8%)	
Cases of pyelitis	34	47	31 (66%)		14 (30%)						2 (4%)	
Cases of cystitis:												
Female	8	11	9 (82%)		2 (18%)						0	
Male	5	8	3 (38%)		3 (38%)						2 (24%)	
Cases of organic obstruction	9	9	3 (33%)		5 (56%)						1 (11%)	
Cases with a neurological lesion	3	5	5(100%)		0						0	
Cases with some structural pathology of urinary tract	24	30	17 (57%)		10 (33%)						3 (10%)	

Out of a total of 165 strains, 22 (12.1%) were not classifiable into any one of the eight groups, and six strains of otherwise typical *Bact. coli*, Type I faecal, did not give a positive methyl-red reaction after three days in glucose-phosphate medium at 37° C. This is a very high proportion, and raises the question of the applicability of this classification to pathogenic organisms—an application it presumably was not designed to have.

When the unclassifiable strains are further examined two groups can be identified, each containing in this series as many strains as or more strains than the three standard types—Irregular Types I and II and *Bact. aerogenes* Type II. The first of these groups consisted of six strains that produced gas at 44° C. but were otherwise typical *Bact. aerogenes*, five being indole-negative and one indole-positive; the second group consisted of seven strains that produced no positive reaction in any of the fluid test media. This latter group was not homogeneous in its other characters. Of the remaining nine strains five liquefied gelatin after varying periods of time but had no other common characteristic, and the remainder was a miscellaneous group. No example of *Bact. coli* Type I failing to produce gas at 44° C. was encountered.

If the intermediate and irregular strains are grouped together they are nearly as numerous as the *Bact. coli* group and five times as numerous as the *Bact. aerogenes* group. This is the grouping that has been used in attempting to correlate types of lesion with their bacteriology and also with the drug sensitivity of the infecting strains.

The time taken in determining the type in the Ministry of Health classification also renders it inapplicable to routine diagnosis. Accordingly those individual reactions used in the classification the results of which may be available in 24 hours, and the appearances of the primary cultures, have been separately correlated with these characters.

44° C. Gas-production Test

Twenty-four-hour readings have been used throughout, as they are the ones that will be available for routine diagnosis, and, in fact, of the first 104 strains none failed to form gas in 24 hours yet did so after 48 hours. On this basis 95 strains produced gas and 70 did not. Of the gas-producing strains 91% showed some sensitivity to penicillin against only 41% of the other strains. The comparable figures for sulphadimidine are 94% and 51% respectively. Both these differences are significant. The behaviour of the strains isolated from infected and contaminated urines was essentially similar. Only 26% of strains that did not produce gas were sensitive to what may be regarded as therapeutic levels of sulphadimidine in non-urinary infections (up to 10 mg./100 ml.).

Performance of the Gas-production Test on the Centrifuged Deposit.—In order to make an early report as informative as possible it is clearly desirable to have the result of this test at the same time as the plate is read. This could be achieved if it were practicable to perform it directly from the specimen. The centrifuged deposits from 48 urines were therefore used as inocula and the results corresponded well with those subsequently obtained from the plate cultures. In order to minimize routine work, it is suggested that this procedure might be performed only on those deposits in which both pus cells and bacilli are seen. On this plan only one discordant result was obtained—that of the primary test being positive while the test performed on what was apparently a pure culture gave a negative result. In such cases the probable mechanism is that a small number of faecal coliforms which are colonially indistinguishable give a falsely positive result. It seems that such cases will be uncommon, perhaps because of the smaller percentage of *Bact. coli* among contaminating strains (See below).

Mucoid Appearance of the Colony

It is well known that some aerogenes strains will, under certain conditions, give a highly typical appearance of gelatinous semi-confluent growth. Mucoid but discrete colonies, on the other hand, although shown by this type, are not confined to it. In all there were 27 strains showing an obviously mucoid colony at 24 hours, 11 of which produced gas at 44° C. Another 17 strains produced an equivocal appearance and have not been further considered. Eight of 89 (9%) *Bact. coli* strains and 7 of 13 *Bact. aerogenes* (54%) were mucoid. There is some correlation between the mucoid characteristic and resistance to 100 u./ml. penicillin although not to sulphonamide. If the strains that are both unable to produce gas and are mucoid are considered it then appears that of 12 strains 7 (58%) are resistant to 100 mg. sulphonamide and 11 (92%) are resistant to 100 u./ml. penicillin. No such strain is either sensitive to 10 mg. sulphonamide or 20 u./ml. penicillin.

Haemolysin; Gelatin Liquefaction

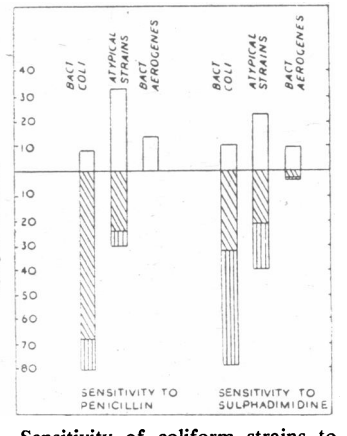
From the first 104 strains examined subcultures were made on to 5% horse-blood-agar plates. It was found that approximately one in three of each type was haemolytic and that this ratio was closely similar in groups of both infecting and contaminating strains. Subsequently the test was not performed.

Only five strains possessed this ability, and none of them was a true *Bact. cloacae* strain. There was no correlation with either the type of the lesion or the sensitivity.

Relation of Strains to Drug Sensitivity

It will be seen from the accompanying Chart that, although the majority of organisms show some sensitivity to both penicillin and sulphadimidine as many as 54 (33%) are insensitive to 100 u./ml. of penicillin. Forty-two (25%) are insensitive to 100 mg./ml. sulphadimidine, and only 64 (39%) are sensitive to ordinary therapeutic levels.

In general, *Bact. coli* strains are sensitive to urinary concentrations of both penicillin (81/89 = 91%) and sulphadimidine (79/89 = 89%), whereas the *Bact. aerogenes* strains are not, four



Sensitivity of coliform strains to 20 u./ml. penicillin and 10 mg./100 ml. sulphadimidine (vertical hatching) and resistance to 100 u./ml. and 100 mg./100 ml. (plain columns). Oblique hatching shows intermediate degrees of sensitivity.

being sensitive to sulphonamide and none to penicillin out of 13.

The atypical coliform strains occupy a position intermediate between the other two groups in this as in other respects (30/63 = 47% penicillin-sensitive and 40/63 = 63% sulphonamide-sensitive).

Relation of Type of Organism to Pathological Features of Lesion

With the exception of pyelitis the number of patients suffering from any one condition is small, but it is possible to group the lesions so that some significant relationships emerge. There is a significantly lower incidence of *Bact. coli* in lesions complicated by organic obstruction (33%) than in pyelitis (66%), as an example of a non-obstructive lesion (difference = 33%, standard error (S.E.) of difference = 16%), and the difference remains significant if the analysis is confined to male cases (difference = 50%, S.E. = 21%). There is also a just significant difference when the obstructive cases (33%) are compared with the remaining infected cases (63%) (difference 30% ; S.E. 15%). It is remarkable, in view of the smallness of the groups, that there are significantly fewer *Bact. coli* in the male (38%) than in the female cases (82%) of cystitis (difference 44% ; S.E. 21%). No such difference was seen in pyelitis nor between cases of pyelitis in pregnant and non-pregnant women. No other significant correlations have been discovered between organisms and lesions, but the number of *Bact. coli* is significantly higher in all infecting strains taken together (60%) than in the contaminating group (41%)—a difference (19%, S.E. = 9%) which is referred to in the discussion.

Cases from which More than One Strain was Obtained

From 22 cases more than one positive culture was obtained. In only 10 of these were the strains of the same type, and it is perhaps worth comment that of five cases of pyelitis from which the same organism was twice obtained it was *Bact. coli* Type I in all. However, in three of the other five cases of pyelitis from which more than one strain was obtained none was of this type.

No definite evidence of change of sensitivity to either penicillin or sulphadimidine was obtained.

Discussion

The findings from this series lend little support to the view (Warner, 1948) that *Bact. aerogenes* is a common invader of the urinary tract, although it must be admitted that the number of neurological and obstructive cases included is small. With the possible exception of the obstructive cases, as opposed to those which merely have some other non-inflammatory genito-urinary abnormality, we have not found the correlation between the precise type of organism and the clinical features of the lesion suggested by Coleman and Taylor (1949). If our cases are classified as were theirs, in Group I, without gross lesions of the urinary tract, 65% are *Bact. coli* (43/66) ; and in Group II, of cases with a surgical or medical lesion, *Bact. coli* are again 65% (22/34). Owing to different methods of presenting results, the relevant literature, mostly referred to in these two papers, is not susceptible to bulk analysis, but we have gained the impression that it leaves this question as open as do our results.

A general differentiation into true *Bact. coli* and the Intermediate-aerogenes-cloacae Group of Wilson and Miles (1946) does, however, possess some clinical and therapeutic applications. It may also be of value in deciding whether a given strain is a pathogen or a contaminant. For routine work such differentiation is best achieved by a careful consideration of colonial appearances and the result of a gas-production test performed on the centrifuged deposit. With this information it is probably justifiable and of value to report the gas-producing non-mucoid strains as *Bact. coli*, the mucoid strains not producing gas at 44° C. as *Bact. aerogenes*, and all others as atypical *Bact. coli*. In this series the percentages then become: *Bact. coli*, 46% ; *Bact. aerogenes*, 8% ; and atypical strains, 46% — the differences between these figures and those in the Table being largely due to inclusion of the mucoid strains of *Bact. coli* among the atypical strains. This merely increases slightly the number of uninformative descriptions while being in no way misleading, and more than half the reports would still carry useful information otherwise not presented.

The relative infrequency of faecal *Bact. coli* and the high proportion of atypical strains among contaminants is at first surprising. It is, however, common laboratory experience that lactose-fermenting organisms presumed to be contaminants often do not "look like" true *Bact. coli* ; and Payne (1949) has shown that these organisms have in fact a short survival time on the skin. This evidence, with that of the rarity of contamination of operating-theatre specimens (Stephens and Enticknap, 1951) and the knowledge that very careful skin-cleansing will do much to eliminate extraneous organisms (Leishman, 1939), explains, in part at least, this finding.

Our sulphonamide and penicillin-sensitivity findings correspond with those of other workers. When these are examined together it is found that of 42 sulphadimidine-resistant strains only two are sensitive to 20 u./ml. and six to 100 u./ml. of penicillin. Thus the sensitivities to the two drugs appear largely to run parallel, and failure of treatment with sulphonamide implies a high degree of probability that penicillin treatment will also fail. In view of the almost universal sensitivity of coliform organisms to streptomycin it does not seem profitable either to investigate their behaviour in relation to penicillin or to exhibit it therapeutically, unless for its action on organisms other than coliforms.

It is clear that *Bact. aerogenes* infections will not respond to sulphonamide treatment, and it is unlikely that half of the infections due to atypical strains will. The outcome of the remaining cases due to strains of intermediate sensitivity is less apparent. The significance of the laboratory finding of sensitivity to high drug concentration, although discussed in its theoretical aspects by Warner (1948), has, so far as we are aware, yet to receive specific evaluation. To obtain clear-cut results of therapeutic and prognostic significance large numbers of cases will require to be analysed because of the numerous independent variables inseparable from routine clinical practice. The method here described is applicable to such a large-scale survey, and with it it is hoped to obtain further information on this and the other points referred to above.

Summary

One hundred and sixty-five strains of lactose-fermenting coliform organisms isolated from contaminated and infected urines have been studied, and an attempt has been made to derive from them a simple classification suitable for routine reporting.

The need for such a simple but informative description based on the data available at the end of 24 hours is stressed, and the unsuitability of the Ministry of Health classification for this purpose is demonstrated.

A routine method for reporting *Bact. coli*, atypical coliform strains, and *Bact. aerogenes* is described, and the significance of this grouping in relation to the penicillin- and sulphonamide-sensitivity is discussed.

No correlation between the type of organism and pathology of lesion, with the possible exception of purely obstructive lesions, was found in this series.

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Medical Memoranda

Two Cases of Locked Twins

The occurrence of the complication of locked twins on two occasions within six weeks is thought to be sufficient reason for reporting this rare event. The cases are of interest because they would seem to support a theory of causation put forward by Lawrence (1949).

Case 1

A primigravida aged 22 was referred to hospital on June 15, 1949, with a diagnosis of breech presentation. She was in the 34th week of pregnancy; the size of the uterus was compatible with the period of gestation, and there was no evidence of toxaemia of pregnancy. The foetal heart could be distinctly heard at two separate points, and the presence of twins was confirmed by *x*-ray examination. The leading foetus presented by the breech and the second by the vertex. Radiological examination of the pelvis showed it to be of the gynaecoid type, the true conjugate being 12 cm.

The further course of pregnancy was uneventful, and the patient was admitted to hospital at 7.30 a.m. on June 29, with the history that labour pains had begun four hours previously. Examination revealed the fundus uteri to be at the level of the ensiform cartilage, and strong contractions were palpable at intervals of ten minutes. There was no evidence of toxaemia of pregnancy, and, as rectal examination showed the cervix to be half dilated, sedation was secured with pethidine hydrochloride, 200 mg. At 2.30 p.m. the membranes ruptured, and twenty minutes later the breech was seen presenting at the vulva.

Pudendal block anaesthesia was carried out with 1% procaine, and when the breech distended the perineum a right medio-lateral episiotomy was performed. The foetus was delivered spontaneously to the inferior borders of the scapulae, and at this level further progress ceased. Examination showed that the head of the second twin was impinging on the neck of the leading one, the head of which was still above the pelvic brim. Under deep general anaesthesia, the head of the second twin was pushed up out of the pelvis and the leading twin delivered by manual extraction. It was found to be a macerated stillborn female child, 17 in. (43.2 cm.) in length and weighing 3 lb. 15 oz. (1,786 g.). As the second foetal heart could not be heard with certainty, internal version was performed and the second foetus delivered by manual extraction. It also was stillborn, and was 16 in. (40.6 cm.) in length and weighed 3 lb. 10 oz. (1,664 g.). Brisk haemorrhage ensued, and the single placenta was manually removed. It was found to have multiple areas of white infarction over two-thirds of its surface. There were two amniotic sacs and one chorion.

Recovery was uncomplicated, and the patient was discharged ten days after delivery.

Case 2

A primigravida aged 25 was referred to the hospital with a diagnosis of plural pregnancy on July 25, 1949. She was then in the 34th week and there was no evidence of toxaemia of pregnancy. Radiological examination confirmed the diagnosis of twins, presenting breech and vertex. The pelvis was of the gynaecoid type, the true conjugate being 12.25 cm.

On August 23 the patient was admitted to hospital, as signs of toxaemia of pregnancy had developed. Her condition improved with rest in bed.

At 12.15 a.m. on September 9 labour pains began and soon very strong contractions were palpable at regular intervals. She was given pethidine hydrochloride, 100 mg., and scopolamine, 1/150 gr. (0.43 mg.), at 4 a.m., when the contractions were recurring at intervals of five minutes. At 6.40 a.m. the membranes ruptured, and at 7 a.m. the breech presented at the vulva. Spontaneous delivery of the foetus ensued to the level of the axillae, when progress ceased. Under cyclopropane anaesthesia the arms were brought down, but the foetus could not be further delivered. Examination showed that the head of the second twin had entered the pelvis with the neck of the first one, the head of which was still above the pelvic brim. The head of the second twin was disengaged and pushed out of the pelvis, and the leading twin delivered by manual extraction. It was a stillborn macerated male child 16 in. (40.6 cm.) in length and weighing 3 lb. 12 oz. (1,700 g.).

Further examination now revealed that the cervix was dilated only to a diameter of four fingerbreadths, and, as the foetal heart was satisfactory, it was not considered necessary to interfere with the course of labour. The second child was delivered spontaneously at 9.5 a.m. in the occipito-posterior position. It was an active male child, 19 in. (48.26 cm.) in length and weighing 6 lb. 10½ oz. (3,019 g.). The single placenta was expelled 20 minutes later; there were two amniotic sacs and one chorion. Recovery was uneventful, and the patient was discharged on the tenth day after delivery.

Comment

The occurrence of locked twins is a rare event: the incidence given by Munro Kerr and Chassar Moir (1949) is 1 in 90,000 deliveries. Several factors have been suggested as causal agents in the production of this complication—namely, small foetuses, large pelvis, deficiency of liquor amnii, mono-amniotic twin pregnancy, and extension of the leading head.

Nicholson (1942) reviewed the causes and put forward the view that, while such predisposing factors might be necessary forerunners to locking, the actual combination of circumstances initiating the mechanism was fortuitous. Lawrence (1949) made a study of available case histories with a view to assessing the frequency of