

## A NEW AND EASY PROCESS OF TRIPLE STAINING FOR CYTOLOGICAL AND HISTOLOGICAL PURPOSES.

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IN the course of an investigation upon the nuclear changes characterising certain new growths I was led into various experimental methods of tissue staining in the hope that I might render my work more easy by devising some simple and rapid way by which a sharp colour differentiation between the various constituents of the nucleus, cytoplasm, and intercellular tissues might be obtained. This object I have attained by a process founded on the "triple stain" of Flemming. An account of it has already appeared in Vol. VI. of the Archives of the Middlesex Hospital and I have since that publication further improved upon it. Flemming's "triple stain" is a method well known to workers in the field of cytology. Its original object was to combine in the tissues the stains saffranin, gentian violet, and orange G. in such a way that the chromatic substance of the nucleus should be stained by the gentian violet, the cytoplasm by saffranin, and the intercellular substances by the orange. This at least appears to have been the aim of its originator, but subsequent workers have not attained this ideal and it has fallen into disrepute amongst certain of them. Thus Bolles-Lee describes it as a "clumsy and uncertain method." It is, however, true that other authorities still warmly commend it, although they do not pretend to realise the ideal of a "triple stain" in the sense of giving three distinct colours. Moreover, even when used as a *double stain* the method is lengthy and uncertain. By the following method a true triple stain can be attained with ease, rapidity, and certainty.

**Technique.**—1. Fix small pieces of the tissue in acetic-alcohol.<sup>1</sup> (The fixatives of Hermann and Flemming may be used if preferred. The older and inferior fixatives such as alcohol, &c., are useless as regards the fine details for which this process was devised, whilst formalin renders it impossible of performance.) 2. Embed, cut, and mount in the usual manner. 3. Stain for one hour in a saturated watery solution of saffranin. 4. Wash in water. 5. Stain for a quarter of an hour in a saturated watery solution of methyl violet. 6. Wash in water and wipe the slide dry except that part occupied by the section. 7. Have ready in a drop-bottle the following solution: to 20 cubic centimetres of acetone add drop by drop a saturated watery solution of orange G. until the flocculent precipitate which slowly appears on shaking is just dissolved in excess of the watery solution; then filter. 8. Flood the slide with this solution. A cloud of colour immediately comes out which obscures the view of the section. 9. Pour this off on to blotting-paper and flood again with the same solution. The colour cloud being much fainter the section can be watched. 10. When the section has attained a *rather faint brownish-pink* colour, pour off the orange-acetone solution. 11. Wash in acetone for a few seconds. (*This should be contained in a small glass jar. Acetone being very volatile, care should be taken that the section does not dry.*) 12. Wash in xylol. 13. Transfer to low-power microscope and see if the proper result has been attained. 14. Wash in two fresh changes of xylol. 15. Mount in xylol-balsam.

**Result.**—All chromatic elements, nucleoli, and certain nuclei, such as those of polymorphonuclear leucocytes, stain a rich violet, chromosomes standing out with peculiar distinctness. The spindle fibres of nuclear mitosis stain a faint pink. The cytoplasm stains a rose pink. The intercellular tissue stains a pale yellow. These effects are best seen if the slide be examined through a deep blue screen.

**Notes.**—A. The method appears to depend upon a selective

action of the orange-acetone on the saffranin and methyl violet. It displaces both of them from the tissues but removes the saffranin most rapidly from the chromatic elements, whilst it displaces the methyl violet most rapidly from the cytoplasm. B. The most important factor in the process is the orange-acetone solution. If this is allowed to act too long the whole section is stained a uniform yellow. If it has not acted long enough the section is blotchy from methyl violet remaining in the cytoplasmic structures and has a distinct violet tint. If the colours are discharged too rapidly the orange acetone is too strong and should be diluted with acetone. The reagent deteriorates after a time. C. The duration of the exposures to the saffranin and methyl violet may be varied respectively according to the particular tissue under preparation. The periods I have given represent a fair average. If the saffranin has acted too long relatively to the methyl violet all the latter stain will be displaced, the saffranin alone remaining in the chromatic elements, a want of distinctness in these structures resulting. If the methyl violet has acted too long the saffranin is discharged from the cytoplasm before the methyl violet and the preparation is blotchy and indistinct. A very slight degree of practice is sufficient to overcome these difficulties. D. If on examination with the low-power microscope (see above) it is found that not sufficient colour has been discharged the treatment with orange-acetone should be repeated. If, however, too much colour has been discharged the specimen had better be taken back from xylol, through acetone, to water, and the process begun over again.

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## THE IMPORTANCE OF A STRICTLY LIMITED LACTATION.

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FOR everyone through whose hands are constantly passing cases of illness in childhood and infancy the question of infant feeding is a matter of primary importance and, consequently, the condition of health of the mother is a subject of the greatest importance and interest. In the study of this question it seemed to me that some interesting light might be thrown on the very complicated subject of the relationship between mother, foetus, and suckling by observations on the concurrence of pregnancy and menstruation with lactation and as a consequence of the results obtained I am led to advance this plea for a more strict limitation of the length of lactation.

My observations were conducted in the case of 100 mothers from whom there were born 405 children (five cases of twins), and of these 400 deliveries there were 374 from which 376 infants survived at least two months. In 259 of these 374 deliveries (69 per cent.) the children were nursed on the breast, wholly or partially, for at least two months; and, therefore, in 259 cases there was a possibility of pregnancy or menstruation occurring during lactation. Of these cases the average duration of suckling was ten months. In six cases the condition as regards the menses was not ascertained, and of the 253 lactations in which this point was determined, in 153 (60 per cent.) there was complete amenorrhœa and in 100 (40 per cent.) menstruation occurred. Of these 100 occasions in which menstruation occurred during lactation the date of the return of menstruation was:—

During the first two months after birth in 27 cases.				
"	third	month	"	" 7 "
"	fourth	"	"	" 4 "
"	fifth	"	"	" 11 "
"	sixth	"	"	" 15 "
"	seventh	"	"	" 9 "
"	eighth	"	"	" 12 "
"	ninth	"	"	" 7 "
"	tenth	"	"	" 1 "
"	eleventh	"	"	" 5 "
"	twelfth	"	"	" 1 "
"	thirteenth	"	"	" 1 "

<sup>1</sup> Pure glacial acetic acid 1 part and absolute alcohol 2 parts. Immerse small pieces of tissue not exceeding a 3 m.m. cube from 5 to 15 minutes, according to size. Dehydrate rapidly in several changes of absolute alcohol. It is of great importance that the glacial acetic acid should be pure (99 to 100 per cent., freezing point 15°C.) and that the alcohol should be absolute.

From which it is seen that in more than one-fourth, or 27 per cent., of these cases menstruation returned within two months of delivery, and in 92 per cent. of cases within nine months, figures closely agreeing with those of Mayer who found that in 685 lactations menstruation returned in 25 per cent. of cases within the first six weeks after delivery.

Of the 253 cases of lactation the position of the children in the family and the condition as to amenorrhœa or menstruation during the lactation, with the average duration of lactation for the children of each position, were as follows (Table I.):—

TABLE I.

Position of child.	Number of cases.	Amenor-rhœa.	Menstruation.	Average duration of lactation.
1st ... ..	71	40	31 (44%)	9 months.
2nd ... ..	54	28	26 (48%)	10 "
3rd ... ..	44	26	18 (40%)	10½ "
4th ... ..	24	15	9 (38%)	11½ "
5th ... ..	22	14	8 (36%)	9½ "
6th ... ..	15	11	4 (27%)	9½ "
7th ... ..	8	7	1 (13%)	12 "
8th ... ..	7	5	2 (29%)	9 "
9th ... ..	4	3	1 (25%)	9½ "
10th ... ..	1	1	0	12 "
11th ... ..	1	1	0	12 "
12th ... ..	1	1	0	12 "
13th ... ..	—	—	—	—
14th ... ..	1	1	0	12 months.

From these figures it would appear that menstruation during lactation is decidedly commoner with the early

children than with the later. This, however, might be due to the fact that the earlier children were longer on the breast, but a glance at the last column of the foregoing table shows that with no child is the average duration of lactation shorter than with the first, and, consequently, that the increased frequency of the return of menstruation during lactation in the earlier lactations is not due to the greater length of the lactation period. As regards the date of the return of menstruation in the cases of the different children in the family, Table II. gives these dates and also the percentage frequency of the return under two months after delivery and at the sixth month:—

TABLE II.

Position of child.	Number of cases.	Return of menstruation.												Under 2 months.	At 6th month.
		Within 2nd month.	3rd month.	4th month.	5th month.	6th month.	7th month.	8th month.	9th month.	10th month.	11th month.	12th month.	13th month.		
1st	31	11	4	2	2	1	5	1	1	1	2	1	—	36	65
2nd	26	5	2	1	3	3	2	6	3	—	1	—	—	19	53
3rd	18	5	—	1	1	3	1	4	2	—	1	—	—	28	56
4th	9	2	—	—	2	3	—	1	—	—	—	—	1	22	89
5th	8	3	—	—	2	1	1	—	1	—	—	—	—	38	75
6th	4	—	—	—	1	2	—	—	—	—	1	—	—	0	75
7th	1	—	—	—	—	1	—	—	—	—	—	—	—	0	100
8th	2	—	1	—	—	1	—	—	—	—	—	—	—	0	100
9th	1	1	—	—	—	—	—	—	—	—	—	—	—	100	100

From the above it is seen that in a considerably greater proportion of cases does menstruation return within six

TABLE III.—SHOWING THE DURATION OF OVERLAPPING OF PREGNANCY AND LACTATION.

No.	Length of lactation.	Date of conception after delivery.	Duration of overlapping of pregnancy and lactation.	Position in family of foetus conceived.	Presence (+) or absence (−) of menstruation before conception.	Age of mother at date of conception.	Condition of children at date of examination.		Number of children in family.
							Suckling.	Fœtus.	
1	11 months.	8 months.	3 months.	Second.	M −	21½ years.	Alive and well.	Alive and well.	4
2	12 "	7 "	5 "	"	M −	29½ "	" "	Died, aged 5 months.	11
3	15 "	10 "	5 "	"	M +	25 "	" "	Phthisical.	8
4	12 "	8 "	4 "	Third.	M +	26½ "	Phthisical.	Delicate.	8
5	12 "	9 "	3 "	"	M +	21½ "	Alive and well.	Stillborn.	3
6	9 "	8 "	1 "	Fourth.	M +	28 "	" "	Miscarriage.	9
7	24 "	18 "	6 "	Third.	M −	30 "	Died, aged 2½ years.	Alive and well.	10
8	24 "	18 "	6 "	Fourth.	M −	32 "	Alive and well.	" "	10
9	11 "	10 "	1 "	Second.	M +	24½ "	" "	" "	3
10	13 "	11 "	2 "	"	M −	20 "	" "	Died, aged 10 months.	4
11	13 "	11 "	2 "	"	M +	23 "	" "	Alive and well.	6
12	11 "	6 "	5 "	"	M −	21 "	" "	" "	9
13	12 "	11 "	1 "	Twins, fourth and fifth.	M +	30½ "	" "	Premature twins; died.	9
14	12 "	8 "	4 "	Third.	M −	24 "	" "	Alive and well.	8
15	15 "	10 "	5 "	Second.	M −	28½ "	" "	" "	3
16	7 "	6 "	1 "	"	M +	23 "	" "	" "	8
17	7 "	6 "	1 "	Third.	M +	24½ "	" "	" "	8
18	10 "	9 "	1 "	"	M −	22½ "	" "	Died, aged 6 months.	14
19	18 "	16 "	2 "	Fourth.	M +	25 "	Delicate.	Miscarriage.	5
20	6 "	4 "	2 "	Second.	M +	20 "	Alive and well.	" "	5
21	9 "	8 "	1 "	Twins, fourth and fifth.	M +	21½ "	" "	Twins—one died at birth; the other very delicate.	5
22	15 "	6 "	9 "	Third.	M +	23½ "	Delicate.	Delicate.	9
23	9 "	5 "	4 "	Second.	M +	20 "	" "	Stillborn.	5
24	13 "	11 "	2 "	Fourth.	M +	35 "	" "	Delicate.	"
25	13 "	10 "	3 "	"	M −	32 "	Alive and well.	" "	5
26	10 "	9 "	1 "	Second.	M −	23 "	" "	Alive and well.	12
27	14 "	12 "	2 "	Third.	M +	25½ "	" "	" "	8
28	9 "	7 "	2 "	Second.	M −	23 "	Died, aged 18 months.	" "	10
29	11 "	8 "	3 "	"	M +	24 "	Alive and well.	Delicate.	5

months in the later children than in the earlier children, and as the average lactation period of the later children was as long as that of the earlier children, it appears that when menstruation occurs in later lactations it occurs early or not at all. Of the 100 mothers examined, 87 suckled a child for two months on at least one occasion, and of these 87 who suckled once or oftener 47 experienced the return of menstruation during lactation; while of the 47, in 26 cases it occurred with every lactation. Menstruation during lactation was thus found to occur in nearly 1 in 2 of the subjects examined and in the great majority of the cases the return of the menses took place within nine months of delivery. That in no single instance was the suckling seriously affected is an important point, and the fact that the return of menstruation is commoner during the earlier than the later lactations would seem to indicate that its return is accelerated in the case of the younger and stronger mothers. This condition, therefore, by no means necessarily serves as a contra-indication for the continuation of suckling, and a French author indeed—M. Gillet—goes so far as to recommend as a wet-nurse the woman who menstruates regularly during lactation, as this he considers to be a sign of robust health.

The condition of pregnancy during lactation is, however, a very different matter. Of my 259 cases where this was a possibility it occurred 29 times, and the following table summarises these cases. Here it will be seen that of the 100 mothers in 24 per cent. pregnancy occurred during lactation and in 29 out of 374 conceptions—i.e., 8 per cent. of conceptions, or in 11 per cent. of cases where the infant was reared for at least two months on the breast. Thus in 24 per cent., or nearly 1 in 4 of the mothers, there occurred at some period an overlapping of pregnancy and lactation, and in five cases this occurred twice, and this occurrence took place in 11 per cent. of the lactations.

From Table III. it is seen that the duration of the overlapping of pregnancy and lactation was:—

In 8 cases ... .. 1 month	In 4 cases ... .. 5 months
„ 7 „ ... .. 2 months	„ 2 „ ... .. 6 „
„ 4 „ ... .. 3 „	„ 1 case ... .. 9 „
„ 3 „ ... .. 4 „	—
	Total 29

and thus that in the majority of cases the period was a comparatively short one. The foetus conceived during lactation was in 14 cases the second member of the family, in eight cases the third, and in seven cases the fourth; and in connexion with later members of a family there was no such occurrence. It therefore certainly appears that this occurrence takes place most frequently in connexion with the earlier children of a family, as might, perhaps, be expected looking to the larger number of early children and the greater frequency of bottle-feeding among the later ones. This would not, however, seem entirely to explain the matter or the entire absence of cases of overlapping among the later children and it may safely be said that this condition of affairs is extremely uncommon. The average duration of lactation of those infants during whose suckling pregnancy occurred was 12 months, while of all the 259 lactations the average duration was ten months. In only two cases did conception occur prior to the sixth month after delivery and the average date of conception was nine months after delivery, while the importance of the lengthened average period of lactation is observed from the fact that no fewer than 12 cases occurred at, or after, the tenth month. These figures largely bear out the statement of McCann that “the greater number of pregnancies during lactation commence after the eighth month.”

In 17 cases (59 per cent.) menstruation occurred prior to conception and in 12 cases (41 per cent.) there was no previous menstruation. Remfry, in the course of 900 lactations, found amenorrhœa in 503, or 57 per cent., and menstruation occurred in 388, or 43 per cent., and these figures correspond very closely with my own, which were 60 per cent. with amenorrhœa and 40 per cent. with menstruation. Of the 503 cases with amenorrhœa he found that pregnancy occurred 29 times (6 per cent. of cases) during lactation, while of the 388 cases with menstruation pregnancy occurred in 226 (60 per cent.) during lactation, and he therefore concludes that where menstruation returns during lactation the chances of pregnancy occurring are ten times greater than where there is amenorrhœa. While in my cases it was also found that pregnancy was more common

after the return of menstruation the same marked disproportion between the two classes of cases was not obtained, and thus while in 153 lactations associated with amenorrhœa pregnancy occurred in 12 cases, or 8 per cent., in 100 cases associated with menstruation pregnancy occurred in 17 cases, or 17 per cent., pregnancy consequently occurring rather more than twice as frequently after the return of menstruation as where there was amenorrhœa.

As regards the condition of the children, those in fairly good health have been described as alive and well, while the weakly children have been described as delicate, the sucklings naturally being of greater age than the foetuses and therefore their lives having been exposed to greater dangers. Notwithstanding this it is found that of the 29 sucklings 22 (76 per cent.) were alive and well at the date of examination, while of the 31 foetuses 13 (42 per cent.) only were alive and well at this date. These figures appear to me to be somewhat striking. The works of Prochownick and Noel Paton, in human subjects and guinea-pigs respectively, have conclusively shown the remarkable effect of variations in maternal diet on the embryos, and one might, *a priori*, expect to find the infant of a poor woman, not too fully nourished and for some period, at least, of her pregnancy engaged in the function of lactation, would start life severely handicapped. That this is so my figures show.

Jacobi, many years ago, stated that “lactation and pregnancy are incompatible,” but this opinion of the condition would appear to have been anything but universally accepted and in support of the contrary opinion Paquy reports 29 cases of pregnancy occurring during lactation in none of which was any evil effect whatever noticed, and he refers to the well-known condition of affairs among domestic animals, an analogy which appears to me in many respects unsound. That the condition is by no means markedly uncommon has already been pointed out by Robertson and Church and the cause of this is undoubtedly to be found in the widespread belief among the working classes that so long as lactation is continued pregnancy will be avoided—a belief which leads inevitably to the undue protraction of breast-feeding. In such cases the importance of the condition is but ill-recognised from the fact that the suckling probably continues well and thriving, while irrevocable injury is being done to the unseen foetus and consequently the greater need there is of emphasising the dangers of the position.

That the constant reiteration of the command for maternal nursing is as imperative to-day as it ever was goes without saying, but it appears to me indubitable that along with this is the very important obligation of the demand for a strictly limited lactation. My own figures are small in number but very largely agree with, and corroborate, those of former authors, and judging by them I advance this opinion on the following grounds: 1. The concurrence of pregnancy with lactation is comparatively common. 2. Conception is rare within the first six months of lactation and uncommon before the eighth month. 3. Where pregnancy and lactation overlap the foetus is likely to suffer. That every case must be judged on its own merits is a truism, but given fairly average conditions, a strong healthy suckling, and the prospect of intelligent hand feeding the above facts point very strongly to the advantages to be derived from weaning early in the eighth month.

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#### TRADE ADVERTISEMENTS AND “THE LANCET.”—

The several correspondents who have sent to us a post-card issued by Messrs. Wright, Layman, and Umney containing an advertisement of Wright's Coal-tar Soap, with a reduced fac-simile of the heading of THE LANCET, will kindly note that this unauthorised and unjustifiable use of our title has been dealt with, and the further issue of the offending card has been stopped. In justice to Messrs. Wright, Layman, and Umney, it should be stated that the card was the creation of their advertising agents who have apologised for the offence and have delivered up to us the remaining stock of the fac-simile headings.