LABORATORY FINDINGS IN ENTERIC GROUP FEVERS IN THE MIDDLE EAST FORCES

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INTRODUCTION

In a hospital receiving large numbers of medical cases from personnel living in the climate and exposed to the conditions prevailing in this part of the world, a constant, though very small, number of cases of the enteric group of fevers is encountered. In dealing with such cases during the last 3 years, a number of observations have been made which were at variance with accepted findings to a greater or lesser degree: some of these were liable to cause confusion in diagnosis.

The laboratory work in connexion with these cases was partly in the early stages for diagnosis and partly in the late stages for clearance. The observations of interest were made mainly in the early stages, and so this article is written chiefly from the point of view of diagnosis.

MATERIAL

The material was collected over a period of 3 years, 1941–3. The cases in the first year were from a small hospital which admitted only British personnel: only eighteen of the series come from this year. The cases in the next 2 years included also Allied and Prisoner-of-War (P.O.W.) patients admitted to a large hospital. Throughout the whole period, the number of cases of enteric fevers in British and Allied personnel remained extremely low, and only one very minor epidemic of paratyphoid A (twenty-two proved cases) occurred. Among the P.O.W.'s there was a marked increase of cases among new prisoners after our second and third (Alamein) advances in the Western Desert, but the increase in each instance lasted for only 2 months—until their inoculation with British T.A.B. vaccine took effect. The numbers of the different types of the group are shown in Table 1.

Table 1. *Enteric group fevers. Numbers encountered in the 3-year period* 1941–3

	British and Allied	P.O.W.	Total	Fatal
Typhoid	65 (6 F.)	28 (7 F.)	93	13
Paratyphoid A	59 (2 F.)	12	71	2
Paratyphoid B	8	39	47	—
Paratyphoid C	2	—	2	—
Clinical or P.M.	23 (1 F.)	2 (1 F.)	25	2
Totals			238	17

ROUTINE INVESTIGATION OF PYREXIAL CASES

In a subtropical area and with large numbers of men living under military conditions, a considerable proportion of hospital admissions is due to short-term fevers, and the cases are usually admitted at an early stage of the disease. The following scheme for investigation of pyrexia with no localizing symptoms or signs was standard and was generally used.

On admission, blood films were examined for malarial parasites. On the second day, if nothing else eventuated, one or more further blood films were taken. On the third day after admission the patient's white blood cells were counted: the result of this was a useful indication as to further procedure, though its diagnostic value was limited. In the great majority of cases the white cell count was within normal limits or slightly lowered. In these cases the clinician decided whether to take a blood culture on that day or the next; if the patient was obviously recovering rapidly, the culture was delayed and often was not required; but if the patient's temperature remained elevated or his general condition was not satisfactory, the culture was taken. Thereafter, further investigations were undertaken in accordance with the course of the disease. If no special symptoms or signs gave the diagnosis, if the patient's temperature continued above normal and if a positive report on the blood culture was not received in 4 days, a repeat blood culture was taken and serum obtained for a Weil-Felix reaction. It was repeatedly found that, during the first week, the general condition of a patient was excellent, though later he proved to be suffering from enteric or typhus fever; the raised temperature, headache, perhaps slightly tender spleen and rash were the only indications of the serious time ahead; the eruption of enteric or typhus might be distinctive, but was not infrequently absent or atypical.

Widal reactions were found to be of no assistance and were discarded from an early date. In many cases not of enteric fevers, false positives were given to both 'H' and 'O' suspensions, on account of T.A.B. inoculations. On the other hand, in a number of cases of enteric fever, 'O' agglutination did not develop throughout the whole course of the disease.

This procedure was subject to modification according to special circumstances, but, as a general rule, it was found to be extremely satisfactory, especially with regard to the results for the enteric fevers.

BLOOD FILMS

Thick films stained by Field's method were used. In four cases in this series (three typhoids and one paratyphoid C), rings, trophozoites and gametocytes of *P. vivax* were found in the first film taken. As no response was obtained to anti-malarial treatment, blood cultures were taken and the enteric organisms isolated therefrom, showing that the anti-malarial treatment was not inhibitory to obtaining enteric organisms from blood cultures.

One patient had malaria 4 months before the onset of his enteric fever, the other three had no previous history of malaria. The presence of the gametocytes in these cases was of interest in several ways. First, it showed that these were not fresh infections with malaria; no cases of fresh double infection with malaria and enteric fever occurred in this series. Secondly, these cases illustrated the point that latent malarial infection was activated by the onset of another febrile disease, in the same way as it can be stirred up by surgical procedures and other agencies. A third point of interest was that it was during the incubation period of the enteric fever that the malarial infection became active, with the result that both infections showed simultaneously or, it might be more accurately stated, the malarial infection was demonstrated sooner than the enteric.

LEUCOCYTE COUNTS

In the investigation of pyrexial cases with no localizing symptoms or signs, the white cell count was not considered to rule out the possibility of enteric fever unless the total and neutrophil count was markedly raised. On the other hand, low total counts with a definite diminution of neutrophil polymorphs were much more commonly found in cases of the common short-term fevers than in enteric fevers.

The average results of the white cell counts, total and differential, done on uncomplicated cases of proved enteric fever in this series are shown in Table 2. As the onset of these fevers is often insidious and the actual date cannot be given exactly, the counts have been divided into 'early' and 'late' as follows: under 'early' are the counts done within the first 7 days of admission to hospital in cases known to have come in soon after the onset; those in the 'late' column consist of counts done on patients known to have been ill for some time before admission, counts done after the patient had been in hospital for more than 7 days and counts done during relapses.

Table 2. *Enteric group fevers. Averages of white cell counts in uncomplicated cases*

	Early							Late						
	No. of counts	Total white cell count per cu.mm.	Percentages Poly. (neutro.)	Lymph.	Mono.	Eosin	Baso.	No. of counts	Total white cell count per cu.mm.	Percentages Poly. (neutro.)	Lymph.	Mono.	Eosin	Baso.
Typhoid	53	5720	63	31	6	.	.	19	6070	60	35	4	1	.
Para A	27	6190	66	27	6	1	.	7	8640	59	35	6	.	.
Para B	17	6220	68	27	5	.	.	9	8000	62	30	6	2	.
All types of proved enteric	97	5940	65	29	6	.	.	35	7070	60	34	5	1	.
Averages for all cases								132	6200	63	31	5	1	.

The extent of the variations met with in the total counts is also shown thus:

W.C.C. per cu.mm.	No. of cases
Under 2500	2
2500–5000	39
5000–7500	64
7500–10,000	19
10,000 and over	8
Total	132

In eight of the counts, the percentage of polymorphs was 80 or over, and in twenty-eight the polymorphs were between 70 and 79 %. The total and differential counts of the eight cases in which the totals were highest are given in Table 3, showing that counts such as these do not preclude the diagnosis of enteric fever; it is noteworthy that

only one of the eight was a typhoid, the others being paratyphoid A and B. In only six cases were total counts of 3000 or lower obtained, and three of these were in typhoid (Table 3).

Table 3. *Enteric group fevers. White cell counts in uncomplicated cases: eight highest and six lowest counts*

No.	Type	Day after admission	Total W.C.C. per cu.mm.	P.	L.	M.	E.
				(percentages)			
1	A	10th	14,350	18	80*	2	.
2	T	3rd	11,800	73	19	8	.
3	A	3rd	11,200	84	12	4	.
4	A	2nd	11,000	80	12	8	.
5	A	1st	10,800	75	18	6	1
6	A	10th	10,800	57	42	1	.
7	B	1st	10,600	80	15	4	1
8	B	6th	10,000	74	23	3	.
9	A	3rd	3,100	61	30	9	.
10	A	3rd	3,000	60	35	3	2
11	T	2nd	2,750	77	18	5	.
12	T	3rd	2,700	51	46	3	.
13	B	4th	2,300	75	20	7	.
14	T	23rd	2,000	24	72	3	1

* Three-quarters of these were 'glandular fever cells'.

These results were most surprising in view of the common text-book statement that enteric fevers cause a leucopenia with a relative lymphocytosis. A small number of cases in this series did show such a picture, but in the great majority of cases the white cell count was towards the lower level of normal limits, both in the initial stages and after the disease had progressed for some time.

Occasionally in the differential counts in this series, typical 'glandular fever cells' were found. The most striking example of this was Case 1 (Table 3), where three-quarters of the 80 % lymphocytes were of this type; the patient showed no signs of glandular enlargement or rash at any stage of his illness. In the few other cases, the proportion of these cells was much lower.

BLOOD CULTURES

Blood culture was the fundamental means of diagnosing the cases in this series (Table 4); in 165 out of 215 proved cases, a positive result was obtained from a culture taken within the first 7 days of the patient's admission to hospital. It was considered that blood culture was always worth trying while the patient was still febrile. If a case of clinical enteric fever relapsed, the diagnosis was occasionally clinched by blood culture at that stage (Table 4), or corroboration given that no other condition was supervening.

Table 4. *Enteric group fevers. Method of diagnosis of 238 cases for the 3 years*

	T.	A.	B.	C.	Totals	
Blood culture:						
Original attack	81	64	37	2	184	
Relapse	2	2	1	.	5	
					—	189
Faeces culture:						
Original attack	5	1	1	.	7	
Relapse	.	.	2	.	2	
Clearance	.	1	4	.	5	
					—	14
Urine culture:						
Original attack	1	3	.	.	4	
Relapse	1	.	.	.	1	
Clearance	1	.	2	.	3	
					—	8
Post-mortem	2	.	.	.	2	
Not cultured	.	.	.	.	2	
					—	4
Clinical:						
Organism not isolated	.	.	.	.		23
Totals	93	71	47	2		238

Method

Each culture was performed in duplicate; 4–5 c.c. of the patient's blood were put into each of two bottles, one containing 25 c.c. of 2 % bile-salt broth, the other the same amount of 1 % glucose broth. The cultures were incubated at 37° C. and, after 1, 3 and 5 days, subcultures were made on MacConkey's bile-salt agar and blood agar plates respectively. Each subculture was kept for 48 hr. before being discarded as sterile. When growth of a Gram-negative organism was obtained from one or both of the plates, several colonies were picked off for fermentation reactions; slide agglutination from the growth on the plate often gave the diagnosis tentatively, but always required sugar reactions and tube agglutination to titre for confirmation. For the fermentation reactions, lactose, glucose and mannite were always used; the presence of motility and inability to form indol were confirmed. When these reactions were correct but no specific agglutination was obtained with unboiled or boiled suspensions from agar slopes, saccharose fermentation was tested and, if absent, daily subcultures were made consecutively on MacConkey plates and agar slopes until the specific agglutination was obtained (see under *Bact. paratyphosum* B below).

A point of great importance was the variety of colonial appearances presented by the enteric group organisms, especially *Bact. typhosum*, on the plate subcultures. It was found essential to make films of every type of colony found on these subcultures and continue the investigation to sugars for every Gram-negative bacillus.

Many of the cultures gave their positive subculture on the first day after the culture was taken,

but a considerable number were sterile on the first and positive on the second subculture. It was also found, on several occasions, that a culture in the bile-salt broth might give a growth (scanty or abundant) of anthracoid organisms or cocci on the first subculture, but, by the second occasion of subculturing, these were in very small number and one of the enteric group had grown up in the meanwhile; this finding was specially noted after the introduction of 2% bile-salt broth, in place of the ½% used throughout the first year. In a few instances, the positive subculture was not obtained until the primary culture had been incubated for 5 days.

A growth of an enteric organism was obtained much more often from the bile-salt broth before any growth came up in the glucose broth, but was sometimes found in the first subcultures from both media: occasionally, the organism was grown only in subculture from the glucose broth, though this was probably due to the fact that the more blood that is taken for culture, the more chance there is of finding the infecting organism, rather than to any special merit in the glucose broth.

Results

The results were assessed on growths from either or both media, i.e. a culture was reported as sterile only if no growth was obtained on subculture from either bottle on each of the three occasions. Out of 1542 cultures during the 3 years, 202 were positive for organisms of the enteric group, 15 for other significant organisms (pneumococci, haemolytic streptococci and staphylococci, etc., and *P. vivax* was found in films from two cultures), 754 were sterile, while the remaining 571 gave growth of a contaminant (chiefly in the glucose broth); the frequency of contaminants is partly explained by the fact that the cultures were sometimes taken under extremely unfavourable conditions, with high winds, in dust storms, etc.

The anthracoid group grew moderately well in the bile-salt broth, but the skin contaminants (diphtheroids, non-haemolytic streptococci and *Staph. albus*) were much more frequently grown in the glucose broth.

In this series of 238 cases of enteric fever, including 23 in which the diagnosis was made on clinical grounds alone, 304 blood cultures were taken with the following results:

Positives		Negatives	Contaminants	
Bact. typhosum	89		*Bact. faec. alk.*	12
Bact. para. A	71		*Bact. coli*	8
Bact. para. B	40		Staphylococci	19
Bact. para. C	2		Diphtheroids	1
			Dust and mixed organisms	22
Totals	202	40		62

Strain variations of the enteric organisms

While full investigation of variations in strains of the enteric organisms was not possible, certain unusual features introduced difficulties in recognition.

Bact. typhosum. Various colonial appearances were presented by this organism on plate subcultures. The usual moist, round, small to medium-sized colony was most commonly found; but the spreading 'vine-leaf' type of colony was occasionally seen, and even large, irregular, opaque forms resembling the anthracoid group occurred.

This organism was often very feebly motile and the motility was observed only at 6–8 hr. but not after 12 hr. incubation in peptone water or fluid sugar media. This point was important in cultures from faeces more than from blood cultures, on account of differentiation from the dysentery organisms.

Fermentation of the appropriate sugars did not always occur in 12–24 hr., so that the sets were always incubated for 36–48 hr. before being discarded as negative.

The greatest difficulty in respect of this organism was the Vi factor which prevented direct agglutination with 'O' and 'OH' antisera. It was found that this factor was more potent on a young agar slope than on an older culture on MacConkey's medium. But slide agglutination from a plate subculture always required confirmation by sugar fermentations and tube agglutination to titre (see under *Bact. paratyphosum* B, below).

Bact. paratyphosum A. This member was by far the most straightforward of the group. Very occasionally it was only very feebly motile. Otherwise, it showed only one main variation—failure to produce gas in fluid sugar media (glucose and mannite), though on stab culture in solid sugar media a very small bead of gas was produced; about twice as many strains showed this variation as produced gas in the fluid media. Agglutination, however, was always clear.

Bact. paratyphosum B. In addition to the common medium-sized, moist, semitranslucent colonies, strains of this organism occasionally showed large, opaque, irregular colonies. Otherwise, cultures of this organism followed the simple rules until the arrival of German P.O.W.'s after the battle of El Alamein. They brought back with them strains in the group phase in which the common somatic antigenic factor with *Bact. typhosum* was very prominent at first. With these strains slide agglutination from the plate subcultures gave a slight reaction with *Bact. typhosum* 'O' and no reaction with specific *Bact. paratyphosum* B antisera; in sugars, acid and gas were obtained with glucose

and mannite; and from the agar slope, no agglutination was obtained with specific antisera; usually, slight agglutination was given with a group serum 'Binns' and complete agglutination with a group *Bact. paratyphosum* B antiserum (only a very small quantity of this was available). On repeated subculture, varying from 7 to 14 times, agglutination with specific *Bact. paratyphosum* B antiserum gradually developed: this change-over to the specific phase was hastened in a few cases by growing the organism on agar in which a trace of the group antiserum was incorporated.

Bact. faecalis alkaligenes. Of the 'contaminants', the finding of *Bact. faecalis alkaligenes* in twelve cultures was of considerable interest, in view of the suggested occurrence of an 'enteric-like fever' due to this organism. The cases of this series in which this organism was obtained from blood culture are considered individually.

Case (*a*). Blood culture on the 17th day gave a growth of *Bact. typhosum*, but before the report was received, a second culture was taken on the 20th day and produced a growth of *B. faecalis alkaligenes.*

Case (*b*). Blood culture on the 4th day gave no growth in 6 days. Blood culture on the 9th day gave *Bact. faecalis alkaligenes.* Blood culture on the 20th day gave *Bact. typhosum.*

Case (c). Blood culture on the 3rd day gave no growth in 6 days. Blood culture on the 9th day gave *Bact. faecalis alkaligenes.* Blood culture on the 20th day gave *Bact. paratyphosum* A.

Case (*d*). Blood culture on the 3rd day gave *Bact. faecalis alkaligenes*, but culture of faeces on the same day gave *Bact. typhosum.*

Case (*e*). Blood culture on the 3rd day gave *Bact. faecalis alkaligenes.* Blood culture on the 7th day gave *Bact. faecalis alkaligenes.* Blood culture on the 11th day gave dust contaminants, and urine culture on the 16th day gave *Bact. paratyphosum* A.

Case (*f*). Blood culture on the 2nd day gave *Bact. faecalis alkaligenes.* Blood culture on the 7th day gave no growth in 6 days. Blood culture on the 11th day gave *Bact. faecalis alkaligenes.* This case was a typical 'clinical enteric'.

Case (*g*). Blood culture on the 3rd day gave *Bact. faecalis alkaligenes.* This was another case with a typical enteric course, including two relapses of 9 and 4 days: the patient came from the same unit as, and at the time of, the mild epidemic of paratyphoid A.

Case (*h*). Three blood cultures, on the 2nd, 9th and 19th days, all gave *Bact. faecalis alkaligenes* and the case, clinically, was one of enteric fever.

The findings in these last three cases suggested the possibility of their conditions being due to *Bact. faecalis alkaligenes.* In these cases, however, investigations of faeces and urines were not thorough; also, the circumstances of case (*g*) and the findings in the former five cases tended to show that the causative organism was one of the enteric group.

FAECES

In the early stages of investigation of pyrexial cases, routine cultures of faeces was not undertaken unless bowel symptoms were present. In the majority of cases of this series, bowel symptoms were absent or constipation was the rule in the early stages. A small number of cases did suffer from diarrhoea, sometimes with blood and mucus in the stool, and a few points of interest arose from the results of these stool examinations.

Eighteen patients with diarrhoea had blood and mucus in the stools which, on microscopic examination, showed inflammatory cellular exudates. In five cases, this was described as *Bacillary Exudate*, i.e. of the cells present apart from red blood corpuscles, over 75 % were polymorphs, the remainder being macrophages, lymphocytes and epithelial cells: this is the picture usually found in the acute stages of bacillary dysentery. The other thirteen cases showed *Indefinite Exudate*—a smaller proportion of polymorphs and more macrophages and epithelial cells. On culture, dysentery bacilli were isolated from only three cases; as the rate of isolation of dysentery bacilli from stools showing these exudates over the period under review was over 50 %, it seemed justifiable to assume that these cases were not all double infections with dysentery and enteric fever and that the enteric organisms could also produce such inflammatory exudates. This assumption was partly corroborated by the fact that the enteric organism was isolated in three cases from straight plating on MacConkey's medium; in several other cases where straight plating was not successful for dysentery bacilli and the clinical course was not typical of dysentery, a later culture using brilliant green enrichment gave the enteric organism. Further corroboration was provided by the finding, in several of the fatal cases, that the enteric lesions were fairly extensive in the colon. In others of the fatal cases, however, an inflammatory cellular exudate was obtained as the disease progressed and, at post-mortem, a non-specific ulcerative colitis was found, in addition to the typical enteric lesions (almost healed in one case).

In cases of pyrexia, clinically suggestive of the enteric group and with early diarrhoea, or when the disease had lasted for 10–14 days and no positive result had been obtained by blood culture, culture of the faeces was carried out, each specimen being plated directly on MacConkey's medium and also inoculated into peptone water with brilliant green in a concentration of 1 in 250,000.* The total number of specimens examined thus in this series (Table 5) was 138, with only twelve positives; of these latter, seven were specimens received within

* Desoxycholate and selenite were not available at the time of these investigations.

the first week of admission. The negatives consist chiefly of stools from the cases finally classified as 'clinical enterics'.

After an enteric fever, six daily specimens each of faeces and urine were cultured and found negative before the patient was discharged from hospital. These 'tests for clearance' were commenced after

Table 5. *Enteric group fevers. Total cultures in 238 cases*

Blood:	Original attack	+ve	195	
		−ve	96	
				291
	Relapse	+ve	7	
		−ve	6	
				13
				304
Faeces:	Original attack	+ve	12	
		−ve	126	
				138
	Relapses and clearances	+ve	34	
		−ve	1438	
				1472
				1610
Urines:	Original attack	+ve	12	
		−ve	93	
				105
	Relapses and clearances	+ve	26	
		−ve	1489	
				1515
				1620

Table 6. *Enteric group fevers. Positive results in tests for clearance in 221 cases*

		No. of specimens	No. of cases	No. of these cases in which a relapse developed	
Faeces	T.	4	3	2	(urines also +ve in these three cases)
	A.	4	4	.	
	B.	22	8	1	
Urines	T.	8	5	2	
	A.	3	2	.	
	B.	9	6	3	

the patient's temperature had been normal for 7–10 days. The brilliant green enrichment method* was used for all specimens and the results are shown in Tables 5 and 6. In those cases in which a positive culture was obtained from either faeces or urine, a week was allowed to elapse and, if the patient did not develop a relapse (as occurred in five cases, in three of which both faeces and urine were positive), the whole series was recommenced. Only two cases, both paratyphoid B, showed any tendency to become chronic faecal carriers; one cleared up after 2 months, the other gave 11 positives over a period of 5 months and was eventually evacuated as a carrier.

* Desoxycholate and selenite were not available at the time of these investigations.

URINES

The culture of urines for enteric organisms, as with faeces, was not done as routine in the early stages. Of 105 specimens examined (using brilliant green enrichment) in the original attack (Table 5), twelve were positive, of which three were specimens received within the first 7 days after admission.

The results of the 'tests for clearance' are shown in Tables 5 and 6 and, though in several cases enteric organisms were isolated two or three times before the six consecutive negatives were obtained, there was no tendency to the chronic urinary carrier state in any cases of this series.

FATAL CASES

The mortality over the whole series was exceedingly low, for which several factors were responsible. The effect of T.A.B. inoculation, the general good health of the Middle East Forces and, by no means least, the nursing treatment all played a part.

By far the great majority of deaths were in true typhoid (Table 1); if cultures had been taken from the two undifferentiated cases, both would probably have proved to be due to *Bact. typhosum*, as both died in the second week from toxaemia. In both the deaths in paratyphoid A, there were prominent complicating factors; one patient also suffered from severe diphtheria, remained extremely toxic throughout and developed pulmonary oedema, dying on the 21st day: in the other case, a severe haemolytic streptococcal infection developed in the throat one month after the onset of the paratyphoid, then an acute colitis supervened with death in the 8th week—at post-mortem, the paratyphoid lesions were almost completely healed, but the whole colon showed a marked acute inflammatory congestion with no actual ulceration.

In the deaths due to typhoid, the higher mortality rate among the P.O.W.'s was explained partly by their different immunization method before capture and partly by the fact that some were in very poor general condition. The causes of death were as follows:

Toxaemia: six cases, including five P.O.W.'s; one died in the 2nd, two in the 3rd, and three in the 4th week of the disease, one with terminal acute colitis in addition.

Perforation: three cases, including one P.O.W.; one died in each of the 3rd, 4th and 5th weeks. In one case, operated upon within 2 hr. of perforation, a hole 0·5 cm. in diameter was found on the anterior wall of the caecum, with numerous very thin floors of ulcers present around both caecum and ascending part of the colon, while no ulcers

were present in the small intestine. In the other two cases, there was peritonitis due to spread of infection through very thin floors of ulcers over considerable areas without any clear-cut perforations.

Haemorrhage: two cases, both in the 3rd week, and both from intestinal lesions, although one case showed numerous toxic haemorrhages in mucous membranes and elsewhere throughout his body.

Colitis: one case, fatal in the 5th week, though small haemorrhages had been occurring for one week before death: in this case, the typhoid lesions had practically healed, but the whole colon showed a very marked hypertrophic ulcerative process in the mucous membrane.

Broncho-pneumonia: one case (P.O.W.), fatal in the 3rd week.

SUMMARY

1. The laboratory findings in a series of 215 cases of enteric fever are described and discussed, with special reference to early diagnosis.

2. In the majority of the cases reviewed, the leucocyte count was about the lower level of normal limits, though considerable variation both upwards and downwards occurred.

3. Blood culture was a very satisfactory and early method of diagnosis; the method used is described fully.

4. Certain variations from normal in the behaviour of the organisms were encountered and are described.

5. *Bact. faecalis alkaligenes* was isolated from blood culture in five cases in which enteric group organisms were also isolated from blood, faeces or urine and in three which were clinically enteric fever. The relation of these observations to the possibility of primary *Bact. faecalis alkaligenes* septicaemia is noted.

6. Seventeen cases were fatal. The causes of death are described.

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