DIFFERENCES IN THE COURSE OF *PLASMODIUM BERGHEI* INFECTIONS IN SOME HYBRID AND BACKCROSS MICE

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In earlier studies it was found that (1) inbred strains of mice differed in their resistance to malaria as determined by their survival after inoculation with *Plasmodium berghei* (Greenberg *et al.*, 1953, 1954); (2) F_1 hybrids of these inbred strains usually survived longer than either parent (Greenberg *et al.*, 1953, 1954; Nadel *et al.*, 1955; Greenberg and Coatney, 1955; Highman *et al.*, 1955); and (3) the backcross of one hybrid (C 57 Black × DBA) to the shorter-lived parent (DBA) was intermediate in survival between the two parents while the backcross to the longer-lived parent (C 57 Black) lived longer than either parent (Nadel *et al.*, 1955). Studies on the pathological changes induced by the parasite (Highman *et al.*, 1955) in short-lived white Swiss mice and long-lived C 57 Leaden × A (LAF) hybrids, revealed the following: (1) The onset of pathological lesions was earlier and the lesions were more severe in the Swiss mice, (2) The cause of death in the short-lived mice was most likely toxemia; anoxia was the most probable cause of death in the long-lived mice. (3) There were differences in the degree to which mature and immature (polychromatophilic) erythrocytes were invaded in the two groups of mice. In regard to the last point, about 84 per cent of the mature erythrocytes of the white Swiss mice were infected on day 8 after inoculation; in corresponding LAF mice only 24 per cent of the mature erythrocytes were infected. The infection of mature erythrocytes declined in LAF mice after day 8 until by the end of the third week only 12 per cent of these cells were infected. The infection of immature erythrocytes was high on the eighth day and continued high until the death of the mice. The proportion of immature to mature erythrocytes increased after the first week until the immature cells constituted about 56 per cent of the population. The infection during the third week in LAF mice was predominantly in immature erythrocytes.

It appeared that there might be some relationship between the degree of infection of mature erythrocytes at the end of the first week and the probability that the mice might die at this time of toxemia. This possibility has been explored in four types of mice which had, in previous experience, distinct differences in mean survival after inoculation with *P. berghei*: (1) white Swiss (about 8 days); (C 57 Black × DBA) × DBA backcross (about 13 days); LAF (about 20 days); and STR × C 57 Black (about 25 days).

MATERIALS AND METHODS

The Kasapa strain of *Plasmodium berghei* was used throughout. This strain was maintained by weekly subinoculation of parasitized blood in general purpose white Swiss mice. White Swiss mice served as donors for all the experiments,

TABLE 1

The age and sex of mice used in the present study

Type of mouse	Age of groups (months)	Sex
White swiss	1½, 2, 2, 2½, 4, 6	5♂ 20♀
C57 leaden × A	7, 10, 12	13♂ 3♀
STR × C57 black	4, 5, 7, 7½, 8	19♂ 6♀
(C57 black × DBA) × DBA	5, 7, 8, 9	19♂ 1♀

and were used when their parasitemia was about 30 per cent. Heparinized whole blood was sufficiently diluted with physiological saline so that each 0.1 ml. contained 1 million parasitized erythrocytes. Recipient mice were given 0.1 ml. of this inoculum through a vein in the tail.

The recipient mice were of four genetic backgrounds and were bred and weaned at the NIH animal breeding facilities. Two of the groups of mice were F_1 hybrids of inbred strains of mice: STR × C 57 Black and C 57 Leaden × A (LAF). One of the groups of mice was the backcross (C 57 Black × DBA) × DBA; these will be called backcross mice for convenience. The white Swiss mice were not inbred. As seen from Table 1, the mice were mature, varying in age from 2 to 12 months, and all but the white Swiss mice were predominantly males. Though the range in ages was large, there were corresponding groups of mice of each genetic extraction of approximately the same age, again with the exception of the white Swiss.

Blood smears were prepared from tail blood and were stained with Giemsa.

RESULTS

In the first series of experiments, the infection in white Swiss mice was compared in turn with that in LAF, STR × C 57 Black, and backcross mice. As a check on reproducibility, the experiment was repeated comparing STR × C 57 Black and white Swiss mice. In this series of trials the following observations were made daily, with a few lapses, on every mouse: (1) total per cent parasitemia, (2) per cent of mature erythrocytes infected, (3) per cent of immature erythrocytes infected, (4) per cent of all erythrocytes which were immature, and (5) total erythrocytes per cubic millimeter. Time of death of each mouse was recorded.

The observations, in the form of mean daily values for one trial of each kind of mouse, are recorded in Figures 1 through 5. In all the mice the infection in mature erythrocytes reached a peak on or about day 7 and thereafter declined (Figure 1).* It is apparent from this figure that the rate of increase of the infection in mature erythrocytes, as well as the highest density observed, varied with the type of mouse. In white Swiss mice about 50 per cent of the mature erythrocytes were infected; in STR × C 57 Black mice less than 10 per cent were infected.

* This figure and the others in this paper appeared in a review article in the Indian Journal of Malariology and are reproduced by permission of the editor of that Journal.

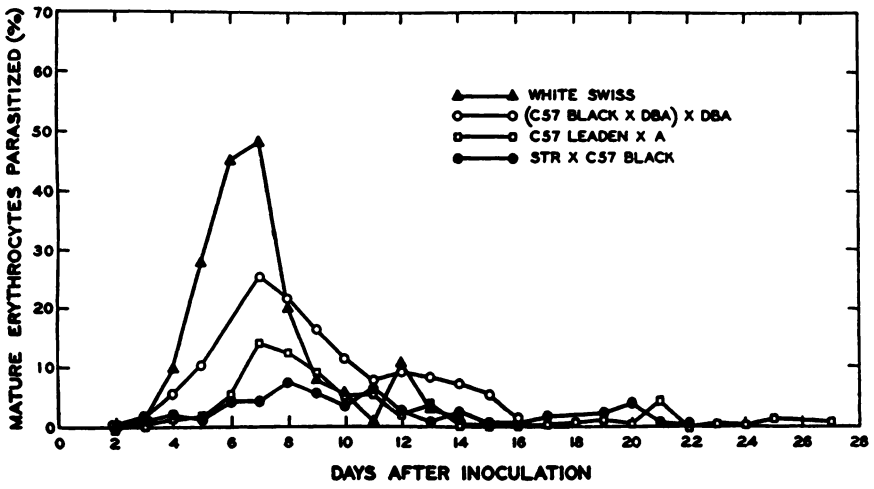

FIG. 1: The course of *P. berghei* infection in mature erythrocytes of four genetically distinct strains of mice.

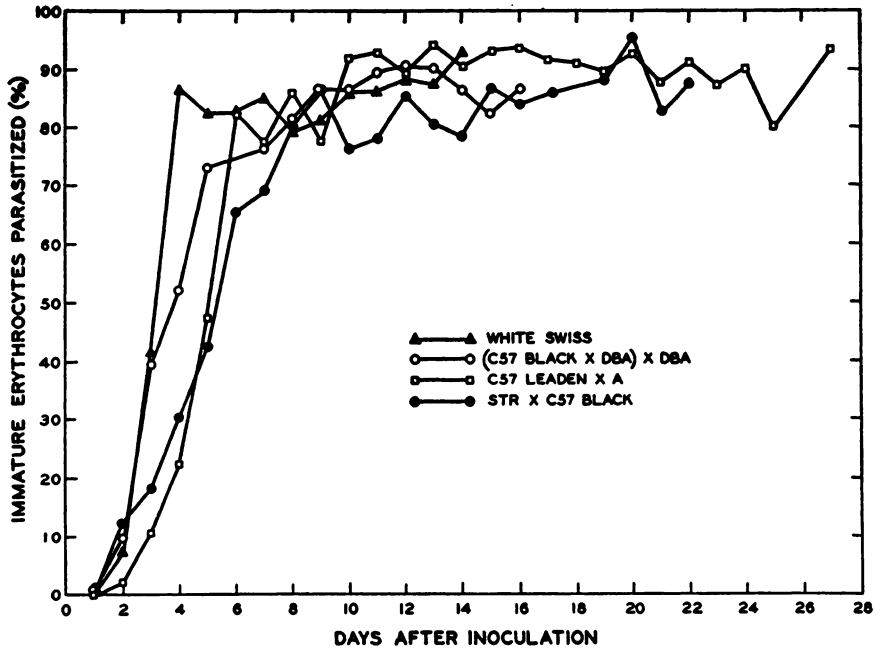

FIG. 2: The course of *P. berghei* infection in immature erythrocytes of four genetically distinct strains of mice.

The infection in immature erythrocytes (Figure 2) rose rapidly in all mice and reached a plateau of between 80 and 90 per cent in 4 to 6 days. There is no indication that in any of the types of mice there was any significant decrease in the per cent infection of immature erythrocytes once a maximum had been reached. The differences among the types of mice during the first week may be real, but

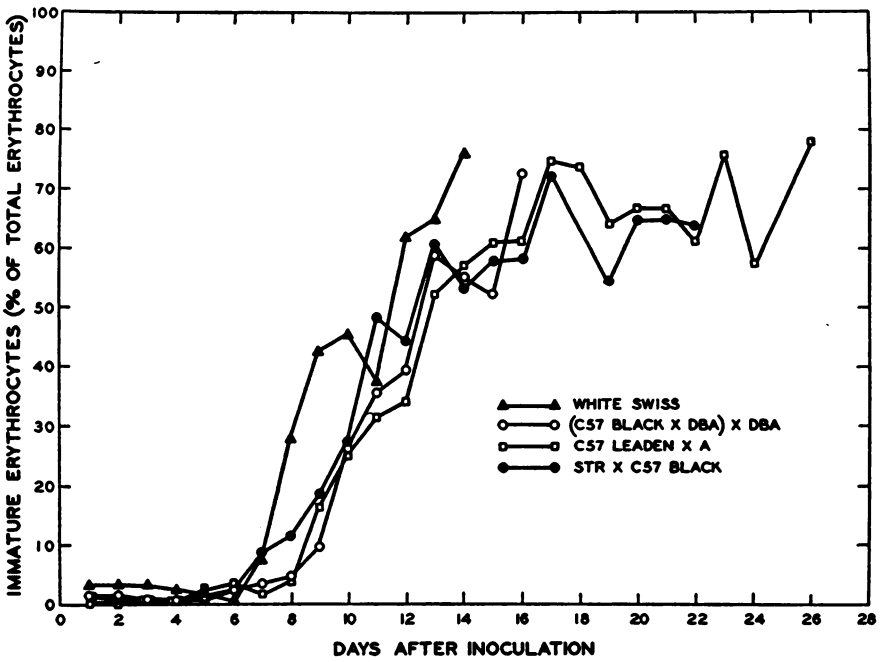

FIG. 3: The proportion of immature erythrocytes to total erythrocytes during the course of a *P. berghei* infection in four genetically distinct strains of mice.

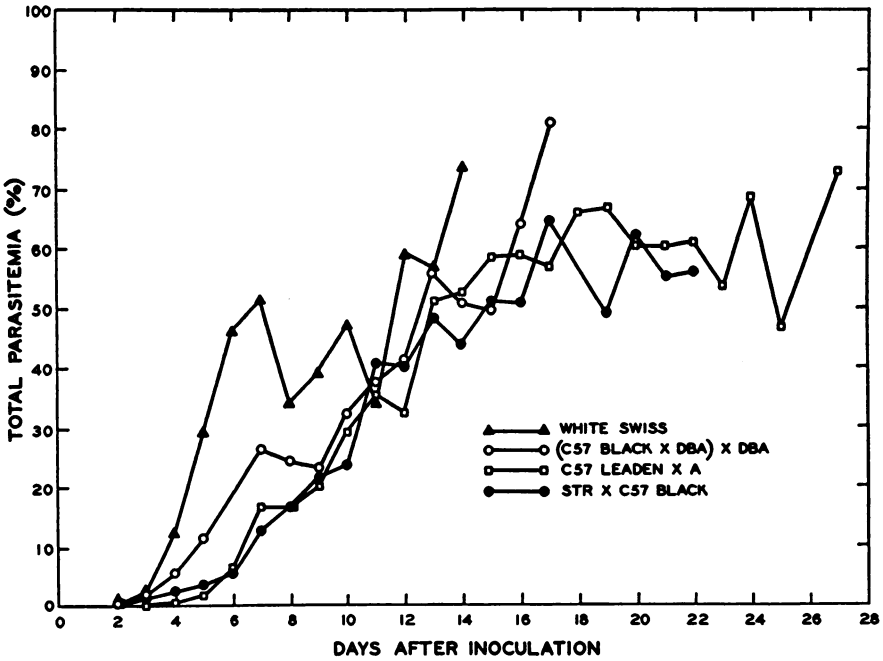

FIG. 4: The course of *P. berghei* infection in all erythrocytes of four genetically distinct strains of mice.

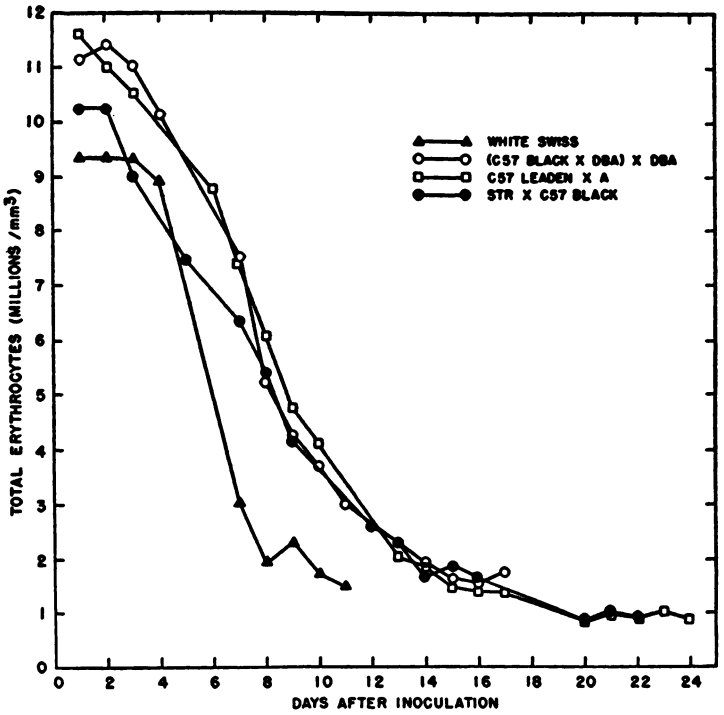

FIG. 5: Total red cell count in four genetically distinct strains of mice infected with *P. berghei*.

they could be attributed to sampling errors with so few immature erythrocytes present in circulation (Figure 3).

During the first week after inoculation, the proportion of erythrocytes which were immature remained low or actually appeared to decline (Figure 3). Thereafter, there was a progressive increase in the proportion of immature erythrocytes until an unstable plateau was reached at the beginning of the second week. The rate of increase in the proportion of immature cells may have been more rapid in the white Swiss mice, but there were probably no significant differences among the other types of mice.

The per cent of all erythrocytes infected (Figure 4) reflects a combination of all the factors described in Figures 1 to 3. The initial peak of infection, distinguishable in some types of mice, can be attributed to the rise and fall in the per cent of mature erythrocytes infected. This initial peak has been described by Schneider and Montézin, 1950; Mercado and Coatney, 1951; Darrow *et al.*, 1952; and by Singer, 1954. The secondary rise in parasitemia can be attributed to the increasing proportion of immature erythrocytes, most of which were infected. In those mice in which there was no decided peak of infection in mature erythrocytes, there was only a vestige of a primary peak of infection.

There is some evidence (Figure 5) that the initial or normal red cell count differs among the types of mice. The erythrocytes per volume began to decline in all mice by the third or fourth day. It dropped more sharply in Swiss mice than

TABLE 2

Mean parasitemia on day seven of four types of mice

Type of mouse	Number of mice*	Mean % infection of mature erythrocytes (day 7)
White swiss	19	50.69 ± 3.99
(C57 black × DBA) × DBA	19	20.83 ± 1.94
C57 leaden × A	16	10.59 ± 1.96
STR × C57 black	24	5.74 ± 1.72

* Differences between these figures and those in Table 1 are mice which failed to survive to day 7.

TABLE 3

Survival of mice in the present studies compared with that of a previous study (Nadel, Greenberg, Jay, and Coatney, 1955)

Type of mouse	Mean survival	
	Present†	Previous‡
White swiss	7.6 ± 0.47	8.4 ± 0.23*
(C57 black × DBA) × DBA	16.55 ± 1.27	13.83 ± 0.48
C57 leaden × A	23.33 ± 1.52	19.29 ± 0.76
STR × C57 black	15.56 ± 0.95	24.45 ± 0.44

* Not previously reported.
† Age given in Table 1.
‡ Age about 2 months ±1 week.

in the others. But among the other types there was a similar rate of decline in erythrocyte numbers.

In the above series of experiments, the most significant factor examined, in terms of the degree of differences and the reliability of the observations, was the course of the infection in mature erythrocytes. Observations on this factor were extended in the following experiments. In this series, representatives of the various types of mice were inoculated at the same time, with the same number of parasites, from the same inoculum. Two observations were made: survival, and the per cent infection of mature erythrocytes. The data from this group of experiments have been pooled with those of the first series and the results of day 7 are shown in Table 2. The contrast in the infection between white Swiss mice and the STR × C 57 Black hybrids is striking; infections in the backcross mice were intermediate between the two extremes. There was a large spread in the data in all groups, and the higher the infection, the larger the spread. Another point of possible significance is the fact that the peak of infection was reached, in general, a day earlier in the Swiss and backcross mice than in the STR × C57 Black and LAF mice. For comparative purposes, the mean infection on day 7 is given in Table 2 for each type of mouse. The difference between the STR × C 57 Black and LAF mice is of no significance ($p < 0.1$), but the differences among all the other types are highly significant ($p < 0.001$).

The survival of all the mice in the present study is given in Table 3. For reference purposes, the survival data for a previous experiment are also given. Most of the latter data have been reported elsewhere (Nadel *et al.*, 1955). All the animals of the previous experiment were approximately two months old. Those in the present series, except the Swiss mice, were 2 to 10 months older. It has been reported (Greenberg *et al.*, 1953) that older mice infected with malaria survive longer than young mice. This could account for the increased survival of the LAF and backcross mice of the present series. The increase in survival in these two types of mice between this and the earlier experiment may be significant ($p < 0.05$). The difference in survival between Swiss mice of the previous experiment and the present one is not significant ($p < 0.2$). The order and the approximate magnitude of the survival data for these three types of mice (Swiss, backcross, and LAF) was approximately the same, considering the age factor, for both series of experiments. On the other hand, the 103 STR × C 57 Black mice which were two months old in the earlier experiments lived significantly longer (9 days, $p < .001$) than the older mice in the present experiment. It is difficult to reconcile this difference with a possible change in the parasite or in technique between the two experiments. It can be tentatively concluded that the rule of increased survival with age is reversed in the STR × C 57 Black hybrid. Such a possibility would be worthy of further investigation.

DISCUSSION

It was pointed out earlier (Nadel *et al.*, 1955) that survival of mice, regardless of genetic background, was bimodal, with one peak of death on day 6 and the other on day 21. It was also observed that a peak of pathological changes, suggestive of acute toxemia, occurred in mice about the end of the first week (Highman *et al.*, 1955). In the present studies it was found that the peak infection of mature erythrocytes occurred on or about the seventh day and that in most animals the maximum decline in red cell count occurred between the sixth and eighth day. The coincidence of these observations: maximum death, peak infection in mature erythrocytes, maximum decline in erythrocyte numbers, and peak pathological changes suggests a causal relationship among these factors. For instance, one could postulate that death at the end of the first week was the result of an acute infection of mature erythrocytes which led to the rapid destruction of mature erythrocytes, and acute toxemia. One would then expect that the higher the peak infection in mature erythrocytes, the more rapid would be the destruction of erythrocytes and the more likely the mouse to die an early death. Such a relationship would appear to be partially true among three of the types of mice used in the present study. The Swiss, backcross, and LAF mice form a consistent series in regard to maximum infection in mature erythrocytes and mean survival. There are, however, certain inconsistencies. The STR × C 57 Black mice are anomalous. On the basis of the rule given above, one would expect them to live as long or longer than the LAF mice. In an earlier experiment with younger mice, they did live significantly longer than the LAF mice. In this experiment (Table 3), they obviously did not. The rule is also of doubtful

validity when one tries to predict the fate of any individual mouse based on the infection of its mature erythrocytes. On the whole, mice with the highest infection of mature erythrocytes died earliest, but those with the lowest infections did not die latest.

But perhaps the rule has the greatest difficulty reconciling the peak infection in mature erythrocytes with the rate of fall in the erythrocyte count. With the exception of the Swiss mice, in which the decline in erythrocyte numbers and the corollary increase in the proportion of immature erythrocytes occurred earlier than in the other mice, the other types of mice were quite similar in these factors.

The coincidence of the factors mentioned above remain, though the explanation of the relationship of the factors is not explained satisfactorily. Evidently, certain data are missing which might tie together death, peak infection of mature erythrocytes, decline in erythrocyte numbers, and pathological changes.

On the other hand, of all the factors studied, infection in mature erythrocytes most clearly differentiates the types of mice studied. This brings up the problem of the preference exhibited by *P. berghei* for immature erythrocytes. This preference has been reported many times (Galliard, 1949; Baldi, 1950; Ramakrishnan and Prakash, 1950; Corradetti and Verolini, 1951; Hsü and Geiman, 1952; Singer, 1953 and 1954). It is apparent from the results of the present experiments that the preference for immature erythrocytes is relative and varies with the strain or type of mouse. It is also apparent that the degree of preference even within a strain varies with the stage of the infection. The preference for immature erythrocytes would be difficult to prove for Swiss mice during the first week of the infection.

It is possible that there is an immunological explanation for the apparent preference of *berghei* for immature erythrocytes; infected mature erythrocytes are removed from circulation more completely and more quickly than infected immature erythrocytes. Certainly, most of those phenomena called immunological have to do with infected mature erythrocytes rather than with infected immature erythrocytes. There is evidence in some types of mice of innate immunity directed toward mature erythrocytes. When compared with the white Swiss mice, the STR × C 57 Black mice would appear to have some mechanism which limits the number of infected mature erythrocytes in the peripheral circulation during the first week of the infection. At the end of the first week there is in all mice which survive long enough, what may be called a crisis, i.e., a rapid destruction, or retention in the deep tissues, of infected mature cells. No comparable events occur in regard to immature erythrocytes; there is no evidence of significant innate immunity and certainly no sign of a crisis. One could assume that the rate of invasion of mature erythrocytes was the same in all types of mice; only the rate of destruction was different. This might account for the fact that the fall in erythrocyte count bears no reasonable relationship to parasitemia. By the first week, mature erythrocytes appear to be removed from the circulation as rapidly in mice with low parasitemia as in those with a high parasitemia. Alternatively, the destruction of mature erythrocytes could be an "all or none" phenomenon, resulting from toxins released by the parasites.

It is obvious that many significant data are missing. The use of different strains of mice and their hybrids has served to point out the gaps in our knowledge of immunity in mice toward malaria. There is evidence which suggests that some of the factors may be genetically controlled. There is also evidence suggesting that mice differ in their ability to survive the acute stress of the infection at the end of the first week. This ability may also be genetically controlled and may be independent of the immunological response of the host to the parasite.

SUMMARY

The following kinds of mice were infected intravenously with *Plasmodium berghei*: white Swiss, C 57 Leaden × A, STR × C 57 Black, (C 57 Black × DBA) × DBA. They were examined daily for total per cent parasitemia; per cent mature erythrocytes infected, per cent immature erythrocytes infected, per cent erythrocytes which were immature, and total red cell count.

There was a peak infection of mature erythrocytes at the beginning of the second week, after which infection in these cells declined to a very low level. The infection in immature erythrocytes rose rapidly in all mice and reached a plateau of about 90 per cent in 4 to 6 days. The proportion of immature erythrocytes to total erythrocytes rose after the first week to a plateau of about 60 per cent. Total parasitemia reflected the initial peak of mature erythrocytes followed by the rise in the proportion of immature erythrocytes, most of which were infected.

The red blood cell count began to drop by the third or fourth day after inoculation. It fell more rapidly in Swiss mice than in the others, but there was little difference among the other mice in this respect.

A more detailed examination was made of the course of the infection in mature erythrocytes. The Swiss mice had a mean count on day seven of 51 per cent; (C 57 Black × DBA) × DBA, 21 per cent; C 57 Leaden × A, 11 per cent; STR × C 57 Black, 6 per cent.

In general, the higher the infection in mature erythrocytes, the shorter lived the mice. There were exceptions and these were discussed. Also discussed was the coincidence of peak infection of mature erythrocytes and the first mode of a bimodal survival curve.

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