Rev. Inst. Med. trop. São Paulo
11(2):94-100, março-abril, 1969

UDC 616.936

COMPARATIVE STUDY OF EXPERIMENTAL INFECTIONS IN MICE INOCULATED WITH NORMAL AND CHLOROQUINE-RESISTANT STRAINS OF *PLASMODIUM BERGHEI*

Antoniana U. KRETTLI, José Pedro PEREIRA and Z. BRENER

SUMMARY

A comparative study has been performed of experimental infections developed in albino mice inoculated with normal and chloroquine-resistant strains of *Plasmodium berghei*.

Although the mice inoculated with the resistant strain have presented, in the late course of infection, parasitemias significantly higher than those displayed by the animals infected with the normal strain, the hemoglobine rates of the two experimental groups were not significantly different. The hematocrit and the mean cell volume, however, have shown to be significantly higher in the mice inoculated with the chloroquine-resistant strain. The causes that could account for the apparent lack of relationship between the parasitemia levels and the hemoglobine rates in the two groups have also been discussed.

Despite the high parasitemias shown be resistant strain, the infected animals have proved to outlive the ones inoculated with the normal strain.

INTRODUCTION

Morphological and physiological differences between normal and chloroquine-resistant strains of *P. berghei* have previously been observed. PETERS [4] reported the absence of ferric pigment in chloroquine-resistant parasites and added that infection with such a strain ran its course more slowly. JACOBS [1] remarked the resistant strain to be considerably less lethal than the parent one. PETERS [6] observed that, in mice infected with the resistant strain, the increase of polychromatophilia kept up with the slow progress of parasitemia, whereas, in the animals inoculated with the normal strain, the infection progressed so fast that the erythropoietic tissue could not meet the challenge. JACOBS & WARREN [2] observed that, in mice inoculated with the resistant strain, the schizonts were seen to be practically restricted to the liver while, in infections with the normal strain, they were found to be either in the circulating blood or in the liver. PALECEK et al. [3] reported more severe anemia, though less intense parasitemia, in mice inoculated with the resistant strain.

This paper presents a comparative study between a normal and a 40 fold chloroquine-resistant *P. berghei* strain, performed through the investigation of some hematological aspects of experimental infection in mice as well as the parasitemia curves and mortality rate. Some aspects such as the relationship between the parasitemia and the hematological picture have been studied in detail.

(Instituto Nacional de Endemias Rurais, C. P. 1743, Belo Horizonte, Brasil)

MATERIAL AND METHODS

P. berghei strains

P. berghei normal strain has been kept in laboratory since 1960 through weekly passages of erythrocytic forms, into mice, by intraperitoneal route.

The method used to induce chloroquine-resistance was based on the work of PETERS [5]. According to this method, a single dose of chloroquine was administered to the mice, by oral route, for four consecutive days, from the day after inoculation. On the 6th or 7th day after inoculation the animals were examined and, when showing parasitemias higher than 3%, sacrificed, their blood being then inoculated into normal mice. In our experiments the first dose selected corresponded to the ED_{50} determined for the normal strain. The other doses were gradually increased, two doses being used in each experiment, the dose administered in the previous treatment, proved insufficient to clear the infected animals, plus a higher dosage. In each passage, three groups of five animals were used: two groups treated with the different dosages of chloroquine and a control group inoculated with the same inoculum, but left untreated.

After inducing resistance to the maximum dosage tolerated by the host (150 mg/kg, oral route), the strain was constantly kept under the action of chloroquine. Blood passages were weekly performed and treatment carried out through four consecutive doses of 150 mg/kg, p.o., beginning from the day after inoculation. The present experiment was undertaken after the resistant strain (CR), had passed into mice for the 75th time.

Inoculation of mice and assessment of parasitemia

18-20 g albino mice were inoculated, per intraperitoneal route, with about 10^7 parasitized erythrocytes. The number of erythrocytes per cmm was determined with a Neubauer chamber, the percentage of parasitized cells being checked out of 300 non-selected erythrocytes counted from smears stained by Giemsa's.

For the assessment of parasitemia the smears were prepared with blood from the mice's tail and examined after staining with Giemsa. The percentage of parasitized red cells was determined out of 500 non-selected cells, using oil immersion.

Dosage of hemoglobin, counting of erythrocytes, determination of hematocrit and mean cell volume (MCV)

Groups of 8 unselected animals were sacrificed on different days after inoculation, their individual blood being collected in tubes containing EDTA. The dosage of hemoglobin was assessed by the method of acid hematin, readings being performed on a Beckman DU spectrophotometer. The counting of red cells as well as the determination of hematocrit and MCV were performed with a Coulter Counter-Model F. In this case the blood was properly diluted to 1:40,000 with an automatic pipette and then submitted to direct reading in the Coulter apparatus.

RESULTS

Tables I and II show parasitemia levels, hemoglobin and hematocrit rates, number of erythrocytes per cmm and the mean cell volume observed both in 77 mice inoculated with *P. berghei* normal strain and in 79 mice infected with chloroquine-resistant strain, examined in different days after inoculation. Figure 1 shows the hemoglobin rates and the percentage of parasitized erythrocytes per each of the 156 mice under experiment. These data show that, in general, mice inoculated with the CR strain display, on the last days of infection, higher parasitemias than those exhibited by the animals infected with normal strain. Actually, the parasitemias observed from the 14th day of infection, in the animals inoculated with the CR strain, were significantly higher ($P < 0.1\%$). In spite of this, no significant difference on the hemoglobin levels were detected during the whole course of infection in the groups of animals inoculated with both CR and normal strains. On the other hand, still on the last days of infection, both the hematocrit and the MCV were also significantly higher in the animals inoculated with the CR strain.

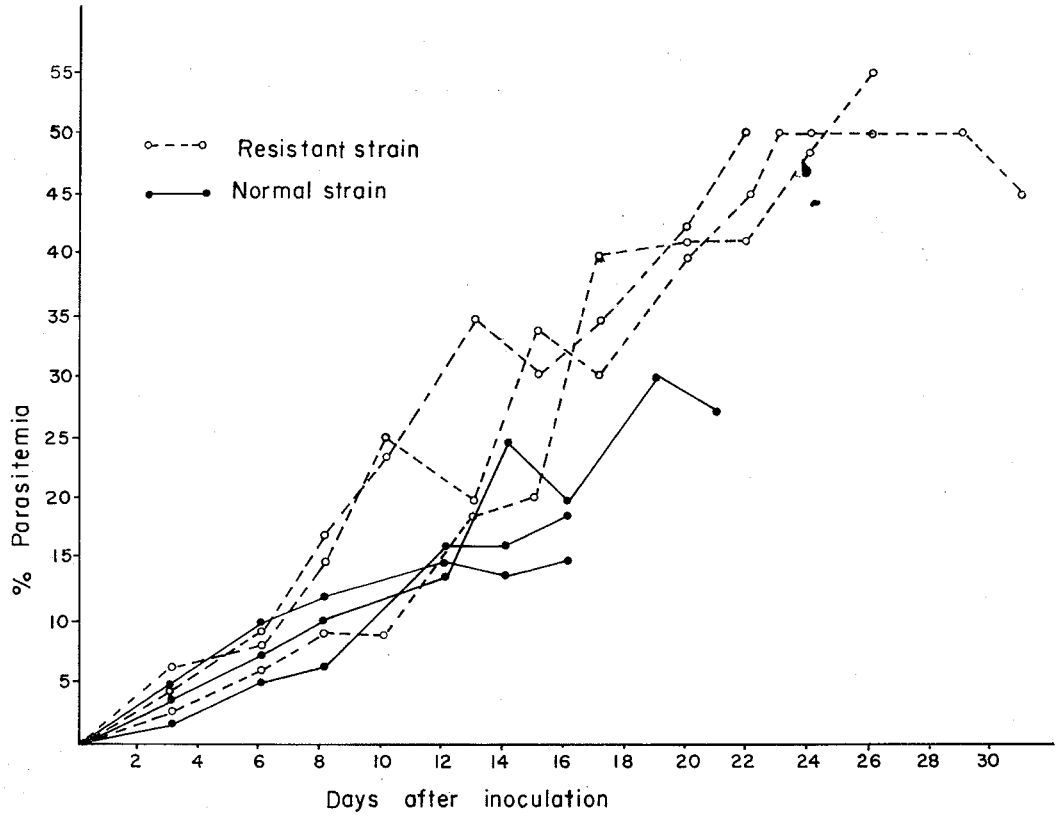

Fig. 2 — Curves of parasitemia in two groups of three mice inoculated with 10^7 infected red cells of a normal and a chloroquine-resistant strain of *P. berghei*.

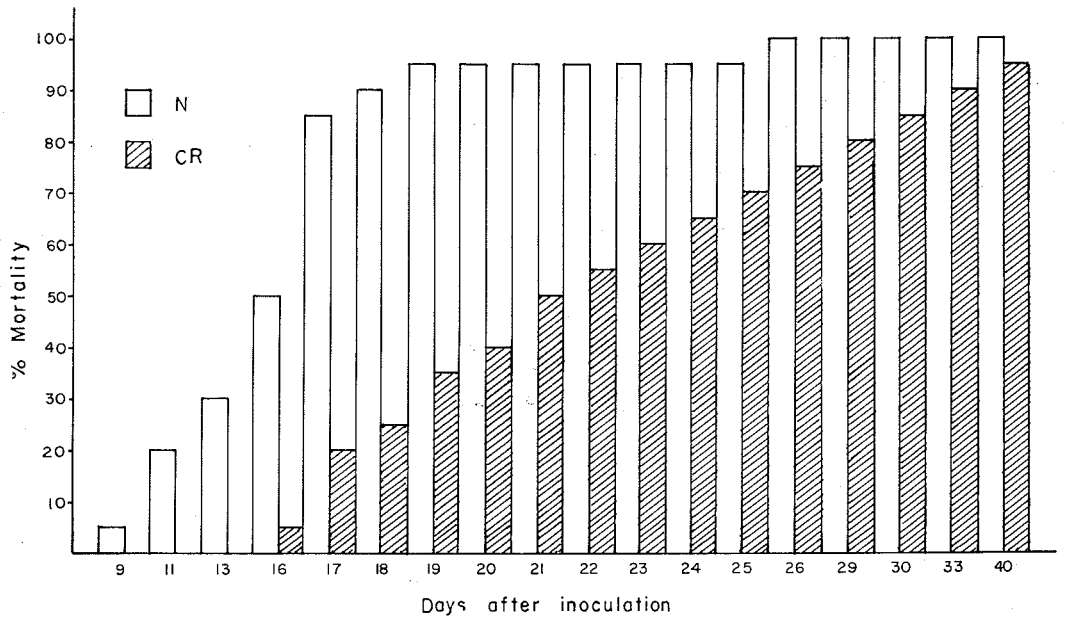

Fig. 3 — Mortality rates in two groups of 20 mice inoculated with 10^7 infected red cells of a normal and a chloroquine-resistant strain of *P. berghei*.

Figure 2 shows the course of infection of two groups of three mice inoculated with normal and CR strain, respectively. All mice were followed up till death, which was rather delayed in the latter group.

Figure 3 shows the mortality rate of two groups of 20 mice infected, per intraperitoneal route, with 10^7 erythrocytes parasitized with normal and CR strains, respectively. A marked delay in the mortality of the group inoculated with the latter strain has been observed.

DISCUSSION

PALECEK et al. [3], investigating the behaviour of mice inoculated with normal and CR *P. berghei* strains, observed that CR strain developed less severe parasitemias but more severe anemias than the normal strain. According to our data, however, most mice inoculated with the normal strain, presenting 20 to 30% parasitemias, meet with an early death caused by severe anemia, whereas experimental infections with CR strain, often displaying parasitemias higher than 40%, present less severe anemia. It is worth remembering that ZUCKERMAN [9] investigating on adult mice the anemia produced by *P. berghei*, reported that blood loss was observed to be markedly higher than that expected from the parasite direct action in the red blood cells and admitted self-immunity mechanisms to be responsible for this phenomenon. This being true such mechanisms seem to be somewhat altered in the infections with CR strain. The difference in behaviour of mice inoculated with N or with CR strain could be largely accounted for the marked increase of polychromatophilia in the latter case and the already known preference of merozoites for these circulating immature cells. Such increase of circulating polychromatophiles occurring with the progress of CR strain infection (PETERS [6]) can be easily detectable by simply examining stained blood smear and, especially, by observing the increase of mean corpuscular volume. By comparing the MCV on Tables I and II we see that, in the end of infection, the figures representing the group inoculated with the CR strain were significantly higher. Our experiments have apparently confirmed PETERS [6] data about experimental infection of normal *P. berghei* progressing so fast that the erythropoietic tissue cannot meet the challenge, whereas parasitemia achieved with CR strain develops slowly, with proportional increase of polychromatophilia. This difference in the behaviour of the hematopoietic system of infected animals may be explained by the presence of iron-containing pigments in the bone marrow of mice inoculated with the normal strain hindering the production of erythrocytes (PALECEK et al. [3]). The well known absence of ferric pigment in CR strain has also been confirmed in our investigation. The significance of the number of reticulocytes present during the course of parasitemia was demonstrated by ZUCKERMAN [8] by studying the anemia caused by *P. berghei* in adult and young rats. As regards the latter animals she reported that immunity was more slowly achieved, the number of reticulocytes in the circulating blood rapidly increased and the parasitemias being more severe frequently caused the animals' death. Through successive bleedings to stimulate erythropoiesis, followed by blood transfusion to compensate for blood loss and, afterwards, by inoculation with *P. berghei*, she was able to reproduce, in adult rats, a malaria clinical picture similar to that observed in young rats. She added that the intense production of reticulocytes led to high parasitemias and that blood loss, in such instances, could be more safely attributed to direct cell rupture by parasites. PALECEK et al. [3], assessing the amount of hemoglobin in the blood of non-infected mice, to which a daily chloroquine dose of 150 mg/kg was given for 6 to 7 days, found it to be higher than that in the blood of untreated control animals. THOMPSON et al. [7] reported the amount of chloroquine in the blood of mice treated with 30 mg/kg, s.c., for seven days, to be as low as 0.6 to 0.9 γ/ml, the assessment having been performed 24 hours after the end of the treatment. It is, however, very hard to assume that this fact could partially explain our results. Actually, the donor mice used in our experiments received four doses of 150 mg/kg, p.o., from the lst to the 4th day, and were sacrificed around the 8th day of infection, their blood being then highly diluted before inoculation in healthy animals.

KRETTLI, A. U.; PEREIRA, J. P. & BRENER, Z. — Comparative study of experimental infections in mice inoculated with normal and chloroquine-resistant strains of *Plasmodium berghei*. *Rev. Inst. Med. trop. São Paulo* **11**:94-100, 1969.

RESUMO

Estudo comparativo de infecções experimentais em camundongos inoculados com amostras de Plasmodium berghei *normais e resistentes à cloroquina*

Foram estudadas comparativamente infecções desenvolvidas em camundongos albinos experimentalmente inoculados com uma cepa normal e uma cepa cloroquina-resistente de *Plasmodium berghei*. Embora os camundongos inoculados com a cepa resistente apresentem, nas fases finais da infecção, parasitemias significativamente mais elevadas que as apresentadas pelos animais inoculados com a cepa normal, as taxas de hemoglobina não apresentaram diferenças significativas nos dois grupos inoculados. O hematócrito e o volume corpuscular médio foram, entretanto, significativamente mais elevados nos camundongos inoculados com a cepa cloroquina-resistente. Foram discutidas as razões que poderiam explicar a aparente ausência de relação entre os níveis de parasitemia e as taxas de hemoglobina nos dois grupos de animais.

Apesar das elevadas parasitemias ocasionadas pela cepa resistente, a sobrevida dos animais infetados é maior do que a dos animais inoculados com a cepa normal.

ACKNOWLEDGMENTS

We wish to thank Dr. Orion C. Bastos for his permission to use the Coulter-Counter of the "Instituto de Previdência dos Servidores do Estado de Minas Gerais".

REFERENCES

1. JACOBS, R. L. — Selection of strains of *Plasmodium berghei* resistant to quinine, chloroquine and pyrimethamine. *J. Parasit.* **51**:481-482, 1965.

2. JACOBS, R. L. & WARREN, M. — Sequestration of schizonts in the deep tissues of mice infected with chloroquine-resistant *Plasmodium berghei*. *Trans. Roy. Soc. Trop. Med. Hyg.* **61**:273-275, 1967.

3. PALÈCEK, F.; PALECEKOVA, M. & AVIADO, D. — Pathologic physiology and chemotherapy of *Plasmodium berghei*. II Oxyhemoglobin dissociation curve in mice infected with chloroquine-sensitive and resistant strains. *Exp. Parasit.* **21**:16-30, 1967.

4. PETERS, W. — Pigment formation and nuclear division in chloroquine-resistant malaria parasites. *Nature* (London) **203**:1290-1291, 1964.

5. PETERS, W. — Drug resistance in *Plasmodium berghei*, Vincke and Lips, 1948. I — Chloroquine resistance. *Exp. Parasit.* **17**:80-89, 1965.

6. PETERS, W. — Morphological and physiological variations in chloroquine-resistant *Plasmodium berghei*, Vincke and Lips, 1948. *Ann. Soc. Belge Med. Trop.* **45**:365-378, 1965.

7. THOMPSON, P. E.; OLSZEWSKI, B.; BAYLES, A. & WAITZ, J. A. — Relations among antimalarial drugs: results of studies with cycloguanil-, sulfone-, or chloroquine-resistant *Plasmodium berghei* in mice. *Amer. J. Trop. Med. Hyg.* **16**:133-145, 1967.

8. ZUCKERMAN, A. — Blood loss and replacement in plasmodial infections. I — *Plasmodium berghei* in untreated rats of varying age and in adult rats with erythropoietic mechanisms manipulated before inoculation. *J. Infect. Dis.* **100**:172-206, 1957.

9. ZUCKERMAN, A. — Autoimmunization and other types of indirect damage to host cells as factors in certain protozoan diseases. *Exp. Parasit.* **15**:138-183, 1964.

Recebido para publicação em 11/11/1968.

Rev. Inst. Med. trop. São Paulo
11(2):101-103, março-abril, 1969

UDC 616.981
616.34-002

ANTIGENIC IDENTITY OF CULTURE 193T-64 AND *E. COLI* 0136:K78(B22)

M. R. F. FERNANDES(1) and L. R. TRABULSI(2)

SUMMARY

By cross agglutination and absorption tests it was shown that culture 193T-64 is identical to *E. coli* 0136:K78(B22), described by SAKASAKI & NAMIOKA in Japan, in 1957. The results are discussed.

INTRODUCTION

In 1964 TRABULSI et al.[7] isolated from a patient with acute enteritis a strain of *E. coli* capable of producing experimental kerato-conjunctivitis in guinea-pigs, which in all aspects was identical to that caused by *Shigella* strains. It was also demonstrated that this isolate was antigenically different from the diverse serologic types of *Shigella* and from the others enteric organisms known to cause experimental kerato-conjunctivitis in the guinea-pig, at that time. For this reason and because it was not possible to determine whether or not it belonged to one of the O antigenic groups of the genus *Escherichia*, the isolate was designated "Culture 193T-64". Organisms identical to culture 193T-64 have subsequently been isolated with relative frequency from the feces of children and adults with diarrhea. Some of these patients presented clinical manifestations typical of bacillary dysentery.

Herein is presented evidence that culture 193T-64 is antigenically identical to *E. coli* serotype 0136:K78(B22) — described by SAKASAKI & NAMIOKA in Japan, in 1957[4].

MATERIAL AND METHODS

Cultures — culture 193-64 was isolated in 1964[7] and the stock strains of *E. coli* 01-0145 were brought by one of us from the Enteric Unit, Communicable Disease Center, Atlanta, Ga. All the strains have been kept in nutrient agar tubes sealed with parafinezed corks.

Anti-sera — *E. coli* 01 to 0145 grouping sera were kindly supplied by the Communicable Disease Center, Atlanta, Ga. OK and O sera for culture 193T-64 and *E. coli* 0136 were prepared according to EDWARDS & EWING[2]. The methods described by the same Authors were followed for the agglutination and absorption tests.

RESULTS

In preliminary agglutination tests using *E. coli* sera 01 to 0145, a heated suspension of culture 193T-64 was significantly agglutinated only by serum 0136. Similarly, when heated suspension of *E. coli* 01 to 0145 were tested, only the *E. coli* 0136 suspension was strongly agglutinated by 193T-64 serum.

Identity of the antigens — When tested in serial dilutions of O antiserum for *E. coli* 0136, the heated suspension of culture 193T-64 was agglutinated to the titre of the serum (1:10.240). Similarly, the heated suspension of *E. coli* 0136 was agglutinated to the titre of the 193T-64 O serum (1:10.240). In addition reciprocal absorption tests showed that the heated suspension of culture

(1) Fellowship of the "Fundação de Amparo à Pesquisa do Estado de São Paulo", Brasil
(2) Professor of Bacteriology (Departamento de Microbiologia e Imunologia da Faculdade de Medicina da Universidade de São Paulo, Brasil). Director: Prof. Carlos da Silva Lacaz

193T-64 removed all the agglutinins from 0136 serum and conversely the heated suspension of *E. coli* 0136 removed all agglutinins from the O serum for culture 193T-64 (Table I).

Further studies using OK sera and living suspensions of *E. coli* 0136 and culture 193T-64, as well as adequated controls to exclude O agglutination, showed that the living suspensions from both cultures were agglutinated to the titre of the heterologous serum (1:160) and that all K and O agglutinins from both sera were removed by the absorbing culture. Ancillary evidence that the K

TABLE I

Comparison of the O antigens of *E. coli* 0136 and culture 193T-64

	O Antisera			
	136		193T-64	
O antigen suspensions (100°C 1 hour)	Unabsorbed	Absorbed by culture 193T-64 (100°C 1 hour)	Unabsorbed	Absorbed by 0136 (100°C 1 hour)
0136	10.240	—	10.240	—
193T-64	10.240	—	10.240	—

TABLE II

Comparison of the K antigens of *E. coli* 0136 and culture 193T-64

	OK — antisera				
	0136:K78(B22)		193T-64		
Antigen Suspension	Unabsorbed	Absorbed by 193T-64 (living)	Unabsorbed	Absorbed by 0136 (living)	Absorbed by 193T-64 (100°C 1 hour)
E. coli 0136 (living)	160	—	160	—	—
E. coli 0136 (100°C 1 hour)	5.120	—	5.120	—	—
Culture 193T-64 (living)	160	—	160	—	—
Culture 193T-64 (100°C 1 hour)	5.120	—	5.120	—	—

antigen of culture 193T-64 was of the B variety was afforded by agglutinin absorption experiments in which OK antiserum 193T-64 was absorbed with a suspension of the homologous culture that had been heated at 100°C for one hour. Since both O and K agglutinins were removed from the antiserum by the heated suspension, it was clear that agglutinin binding power of the K antigen was not destroyed and hence, that the K antigen was of the B variety (Table II).

From the above results it may be concluded that both the O and K antigens of culture 193T-64 are identical to the O and K antigens of *E. coli* 0136:K78(B22).

DISCUSSION

It has been demonstrated that several *E. coli* strains share with *Shigella* serotypes the capacity of causing experimental kerato-conjunctivitis in the guinea-pig. Such *E. coli* strains have been found in O groups 25, 28, 32, 42, 124, 143 and 144 [1, 3, 5, 6, 8]. To these series now *E. coli* 0136:K78(B22) can be added.

Most of the *E. coli* cultures capable of causing experimental kerato-conjunctivitis in the guinea-pig have been found in association with cases of human enteritis. Similarly the six strains of *E. coli* 0136 studied by SAKASAKI & NAMIOKA were all isolated from patients with diarrhea and the feeding of one adult volunteer was followed by severe enteritis [4]. Data on the importance of this bacteria as a cause of enteritis in this country and on its experimental pathogenicity for man, will be presented in a further paper.

RESUMO

Identidade antigênica da cultura 193T-64 *e* E. coli 0136:K78(B22)

Por meio de provas de aglutinação e de absorção cruzadas, demonstrou-se que a cultura 193T-64 é idêntica à *E. coli* 0136:K78 (B22) — descrita por SAKASAKI & NAMIOKA, em 1957, no Japão. Os resultados são discutidos.

ACKNOWLEDGMENTS

Acknowledgments to the "Instituto Brasileiro de Estudos e Pesquisas de Gastroenterologia" — IBEPEGE — for the financial support.

REFERENCES

1. ALDOVÁ, E. — Comunicação pessoal, 1967.

2. EDWARDS, P. R. & EWING, W. H. — *Identification of Enterobacteriaceae.* 2nd. Edition. Atlanta, Burgess, 1962.

3. FERNANDES, M. R. F. & TRABULSI, L. R. — A new *Escherichia coli* serotype causing experimental kerato-conjunctivitis in the guinea-pig (Culture 412-DEC-66). (Preliminary report). *Rev. Inst. Med. trop. São Paulo* **9**:62, 1967.

4. SAKASAKI, R. & NAMIOKA, S. — Studies on a new *Escherichia coli* type: 0136:K78 (B22). *Japan. J. Exp. Med.* **27**:411-416, 1957.

5. SERÉNY, B. — Biochemical reactions and virulence of *E. coli* 0124:K72(B17). *Acta Microbiol. Acad. Sci. Hung.* **10**:11-18, 1963.

6. TRABULSI, L. R.; FERNANDES, M. R. F. & ZULIANI, M. E. — Novas bactérias patogênicas para o intestino do homem. *Rev. Inst. Med. trop. São Paulo* **9**:31-39, 1967.

7. TRABULSI, L. R.; ZULIANI, M. E. & SERRANO, J. A. — On two new enterobacteria pathogenic to the guinea pig eye (Cultures 185T-64 and 193T-64). *Rev. Inst. Med. trop. São Paulo* **7**:241-246, 1965.

8. VÖRÖS, S.; RÉDEY, B. & CSIZMAZIZ, F. — Antigenic structure of a new enteropathogenic *E. coli* strain. *Acta Microbiol. Acad. Sci. Hung.* **11**:125-129, 1964.

Recebido para publicação em 28/10/1968.