قسم المراقبة الصحية
كلية الطب البيطري ـ جامعة أسيوط
رئيس القسم : أ٠د/ توفيق البسيوني

نمو ميكروبات اليارسينيا الممرضة أثناء حفظ الاغذية

مصطفى خليل ، طلعت الخطيب ، أحمد عبدالحميد

اختبرت مقدرة عترتين ممرضتين من ميكروب اليارسينيا (E675 Serotype O : 3) (2635 Serotype O:8) بعد حقنهما معا في اللحم المفروم والسجق الطازج واللبن المبستر وكذلك الآيس كريم وحفظت عبنات اللحم والسجق عند درجات ٢٥ ، ٤ ، صفر ، ـ ٢٠م وعينات اللبن عند ٢٥ ، ٤م أما عينات الآيس كريم فقد حفظت عند درجتي صفر ، ـ ٢٠م وبالكشف الدوري على :

الاغذية المحفوظة عند درجة ٢٥م بعد صفر ، ٦ ، ١٢ ، ١٨ ، ٢٤ ، ٤٨ ساعة ٠

والمحفوظة عند درجة ٤م بعد صفر ، ١ ، ٣ ، ٥ ، ٧ يوم ٠

والمحفوظة عند درجة صفر م بعد صفر ، ١ ، ٢ أسبوع ٠

والمحفوظة عند درجة ـ ٢٠م بعد صفر ، ١ ٢ ، ٣ ، ٥ أسبوع ٠

لمعرفة العدد الكلي للميكروبات وعدد ميكروب اليارسينيا تبين أن هذا الميكروب ينمو بزيادة كبيرة في الاغذية المحفوظة عند درجتي ٢٥ ، ٤م وكانت الزيادة طفيفة عند درجة صفرم بينما نقص في الاغذية المحفوظة عند ـ ٢٠م ٠

وقد تم مناقشة الاهمية الصحية لوجود هذه الميكروبات وسمومها على الصحة العامة ٠

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DEVELOPMENT OF VIRULENT *YERSINIA ENTEROCOLITICA* IN FOOD AT DIFFERENT STORAGE TEMPERATURES

(With One Table & 4 Figs.)

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SUMMARY

Ground meat, fresh sausage, pasteurized milk and ice cream were experimentally contaminated, using a combination of two virulent Yersinia enterocolitica strains, and stored at different temperatures. When inoculated ground meat, sausage and pasteurized milk were stored at 25°C (0 - 48h) or at 4°C (0 - 7 days), large increase in Y. enterocolitica count occurred. Extensive reduction in the counts of Y. enterocolitica occurred during frozen storage (-20°C) of inoculated ground meat and sausage, while a slight decrease was noted with inoculated ice cream samples. Public health implications of these findings are discussed.

INTRODUCTION

Yersinia enterocolitica infections in human have been recognized with increasing frequency in recent years. Gastroenteritis is the most common clinical association, particularly in young children (DELMORE, et al. 1974 and KOHL, et al. 1977), pseudoappendicitis (JEPSEN, et al. 1976), non suppurative arthritis (WINBLAD, 1975) are also described. Although the epidemiology of Y. enterocolitica is not fully established, contact with infected animals, person to person transmission within an infected family or consumption of contaminated food have been mentioned as modes of transmission (GUTMAN, et al. 1973).

Y. enterocolitica has been isolated from various foods, including beef, pork, milk and ice cream (LEE, 1977). It has also been proposed as possible agents of toxin production in food (BOYCE, et al. 1979; FRANCIS, et al. 1980 and KAPPERUD and LANGELAND, 1981). Available data indicate that Y. enterocolitica enterotoxin may be able to resist the temperatures used in food processing and storage (BOYCE, et al. 1979). Large outbreaks of foodborne yersiniosis in the U.S. (BLACK, et al. 1978), Czechoslovakia (OLSOVSKY, et al. 1975), Japan (ZEN-YOJI, et al. 1973) and in Canada (Health and Welfare, Canada 1976) have led to world-wide investigations of foods as a vehicle of Y. enterocolitica infections.

Since, there are very few published reports on the survival of Y. enterocolitica in Egyptian foodstuffs (AHMAD, et al. 1986), the purpose of this study was to evaluate the role of storage temperatures in controlling the growth of virulent Y. enterocolitica in ground meat, sausage, pasteurized milk and ice cream.

MATERIAL and METHODS

Cultures:

The two Y. enterocolitica cultures used in this study included a virulent strain, (3675 serotype 0:3) and the strain (2635 serotype 0:8), that caused the chocolate milk outbreak. These strains were obtained from the Food Research Institute, University of Wisconsin, Madison, U.S.A pool of the two strains was formed from individual 18 h cultures grown at 25 C in brain heart infusion broth (Oxoid) before it was used as inoculum.

Food samples:

The following foodstuffs were chosen primarily because of their previous implications in food-borne outbreaks.

- Ground meat: red cattle meat, obtained from Assiut slaughter house was ground in the laboratory under sterile conditions.
- Sausage: Egyptian fresh sausage was prepared to contain 77% meat, 20% fat, 1% spices and 2% NaCl by the method described by EL-KHATEIB and ABDEL-RAHMAN (1987).
- Pasteurized milk: Whole raw milk was collected from the farm of the Faculty of Agrc., Assiut Univ., and pasteurized in the laboratory by heating in thermostatically controlled water bath at 63°C for 30 min.
- Ice cream: Ice cream samples in their containers were obtained from a local grocery.

Samples of ground meat, sausage and ice cream were screened for Y. enterocolitica by enrichment at 4°C and KOH postenrichment treatment (AULISIO, et al. 1980) and stored at -20°C. Those samples confirmed as negative for the organism were used for inoculation studies.

Inoculation:

Enough of the pool culture was added to the well mixed food sample to provide about 10^5 cells/g or ml. After thorough mixing, inoculated ground meat and sausage samples were stored at 25, 4, 0 and -20°C; pasteurized milk at 25 and 4°C; while ice cream samples were held at 0 and -20°C.

Enumeration procedures:

Food samples and their dilutions were surface plated onto Cefsulodin Irgasan Novobiocin (CIN) plates (SCHIEMANN, 1979) for Y. enterocolitica counts, and onto Standard Plate Count (SPC) agar plates (MARTH, 1978) for aerobic plate counts. CIN plates were incubated at 25°C for 48h. Identification of typical colonies presumed to be Y. enterocolitica was done using the API-20E strips (Analytab Products, Plain-View, NY). SPC plates were incubated at 32°C for 48h. Cell numbers were determined at 0, 6, 12, 18, 24 and 48h for foodstuffs stored at 25°C; at 0, 1, 3, 5 and 7 days for those held at 4°C; at 0, 1 and 2 weeks for those stored at 0°C; and at 0, 1, 2, 3 and 5 weeks for foods stored at -20°C.

RESULTS

The obtained results are illustrated in figures 1-4 and Table (1).

DISCUSSION

Growth of the two pooled strains of Y. enterocolitica in ground meat, fresh sausage and pasteurized milk held at 25°C as calculated from confirmed colonies on CIN plates is presented in Fig. (1). Increases in Y. enterocolitica in these foodstuffs after 24h. were 2.1, 0.8 and 3.4 logs, and after 48 h 2.8, 1.7 and 4.3 logs, respectively. Aerobic plate counts (APC) of inoculated foodstuffs after inoculation and during storage are summarized in Table (1). The means of APC per g or ml. of ground meat, sausage and pasteurized milk after inoculation were $4x10^7$, $6x10^6$ and $5x10^6$ cells; after 48 h at 25°C were $7x10^{10}$, $1x10^{11}$ cells, respectively.

The initial Y. enterocolitica inoculum developed 3.1, 1.8 and 3.4 logs in ground meat, sausage and pasteurized milk samples stored at 4°C for 7 days, respectively (Fig. 2). The APC in these foodstuffs averaged $3x10^9$, $1x10^8$ and $1x10^{10}$, respectively, at that time.

The growth of Y. enterocolitica in above foodstuffs at 4°C reflects the psychrotrophic capability of the organism and makes it unique among enteropathogens. The data of Y. enterocolitica development in ground meat are in general agreement with observation by HANNA, et al. (1977 a). In the same year, HANNA, et al. (1977 b) showed that Y. enterocolitica could be detected even in the presence of large numbers of competing microorganisms. Similar results were obtained in this study. STERN, et al. (1980) indicated that Y. enterocolitica has the capability for growth in milk at refrigeration and room temperatures, but is poor competitor with common spoilage organisms. The differences in counts of Y. enterocolitica obtained in this investigation may have been caused by differences in the level and type of microbial flora that developed on ground meat, sausage and pasteurized milk during storage.

In general, growth of Y. enterocolitica in ground meat and sausage during the first 7 days was considerably higher at 4°C than at 0°C (Fig. 2 & 3). Increases in Y. enterocolitica counts in ground meat, sausage and ice cream after 2 weeks at 0°C were 1.3, 0.3 and 1.4 logs, respectively.

In subsequent trials, ground meat, sausage and ice cream samples inoculated with Y. enterocolitica were stored at -20°C for 5 weeks. Extensive reduction in the counts of Y. enterocolitica occurred during frozen storage (-20°C) of inoculated ground meat and sausage samples, while, a slight decrease was noted with ice cream stored at the same temperature (Fig. 4). 2.3, 3.1 and 0.6 logs reduction in the counts of Y. enterocolitica inoculated into mentioned foodstuffs were observed after 5 weeks when the initial inoculums were $4x10^5$, $2x10^5$ and $3x10^5$ cells/g., respectively.

It is clear from the foregoing that Y. enterocolitica were found to have survival properties in ground meat similar to those reported by EL-ZAWAHRY and ROWLEY (1979). They found that when ground beef inoculated with Y. enterocolitica was stored at -20°C for 30 days, aproximately 83% of the inoculated cells were destroyed. HANNA, et al. (1977 c) also reported both injury and inactivation of Y. enterocolitica in beef stored at -18 to -20°C.

In this respect, reports concerning the growth and survival of Y. enterocolitica in sausage and ice cream is still opened for further investigation. However, the organism has been isolated from ice cream by some investigations (MOLLARET, et al. 1972 and DELMAS and VIDON, 1985).

In one study involving human volunteers, an oral dose of $3.5x10^9$ Y. enterocolitica was needed to provoke diseae (MORRIS and FEELEY, 1976). Based on the above study and assumption that at least 100 g. or more of ground meat or sausage are consumed at one time, then

about 10^7 Y. enterocolitica/g. are needed to cause illness. The same will occur if 200 ml. of pasteurized milk are consumed. In this study, Y. enterocolitica may reach infective level in ground meat, sausage or pasteurized milk within a few days at 25°C as well as 4°C.

Certain types of fermented sausage, such as some dry sausages are not heat treated (KRAMLICH, 1978). In addition, if contaminated foodstuffs serve as an ingredient in certain other foods, this may present a potential problem. Other investigators have found that Y. enterocolitica can survive during refrigerated storage in water for at least 6 months (HARVEY, et al. 1976).

Storage of ground meat, sausage and ice cream at -20°C (Fig. 4) controlled the growth of virulent Y. enterocolitica and kept them below the 10^7 cells/g. level. This is a substantial contribution to the safety of storage of foodstuffs at deep freezing temperatures.

In conclusion, the results presented in this study indicated that Y. enterocolitica can grow to high and therefore presumably hazardous levels in foodstuffs stored at domestic refrigeration temperature (4 C). In a warm atmosphere (25 C), if the foodstuff is kept for a prolonged period (24-48h.), could be a hazard. Presence of even small numbers of virulent Y. enterocolitica in a foodstuff presents a potential health hazard and should not be ignored.

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Table (1)
Aerobic plate counts of stored food samples at different temperatures

Food	hours / 25 C						days / 4 C				weeks / 0 C			weeks / -20 C			
	0	6	12	18	24	48	1	3	5	7	0	1	2	1	2	3	5
Ground meat	$4x10^7$	$2x10^8$	$8x10^8$	$1x10^9$	$5x10^{10}$	$7x10^{10}$	$1x10^8$	$4x10^8$	$2x10^9$	$3x10^9$	$4x10^7$	$1x10^8$	$2x10^8$	$4x10^8$	$2x10^8$	-	$5x10^7$
Sausage	$6x10^6$	$1x10^7$	$5x10^8$	$3x10^9$	$2x10^{10}$	$1x10^{11}$	$5x10^7$	$3x10^7$	$2x10^7$	$1x10^8$	$6x10^6$	$4x10^7$	$6x10^7$	$1x10^8$	$1x10^7$	-	$6x10^6$
Pasteurized milk	$5x10^6$	$2x10^6$	$2x10^7$	$7x10^7$	$2x10^8$	$5x10^{10}$	$3x10^7$	$2x10^9$	$3x10^{10}$	$1x10^{10}$	-	-	-	-	-	-	-
Ice cream	-	-	-	-	-	-	-	-	-	-	$5x10^7$	$2x10^8$	$2x10^8$	$3x10^7$	$2x10^7$	$1x10^6$	$1x10^6$

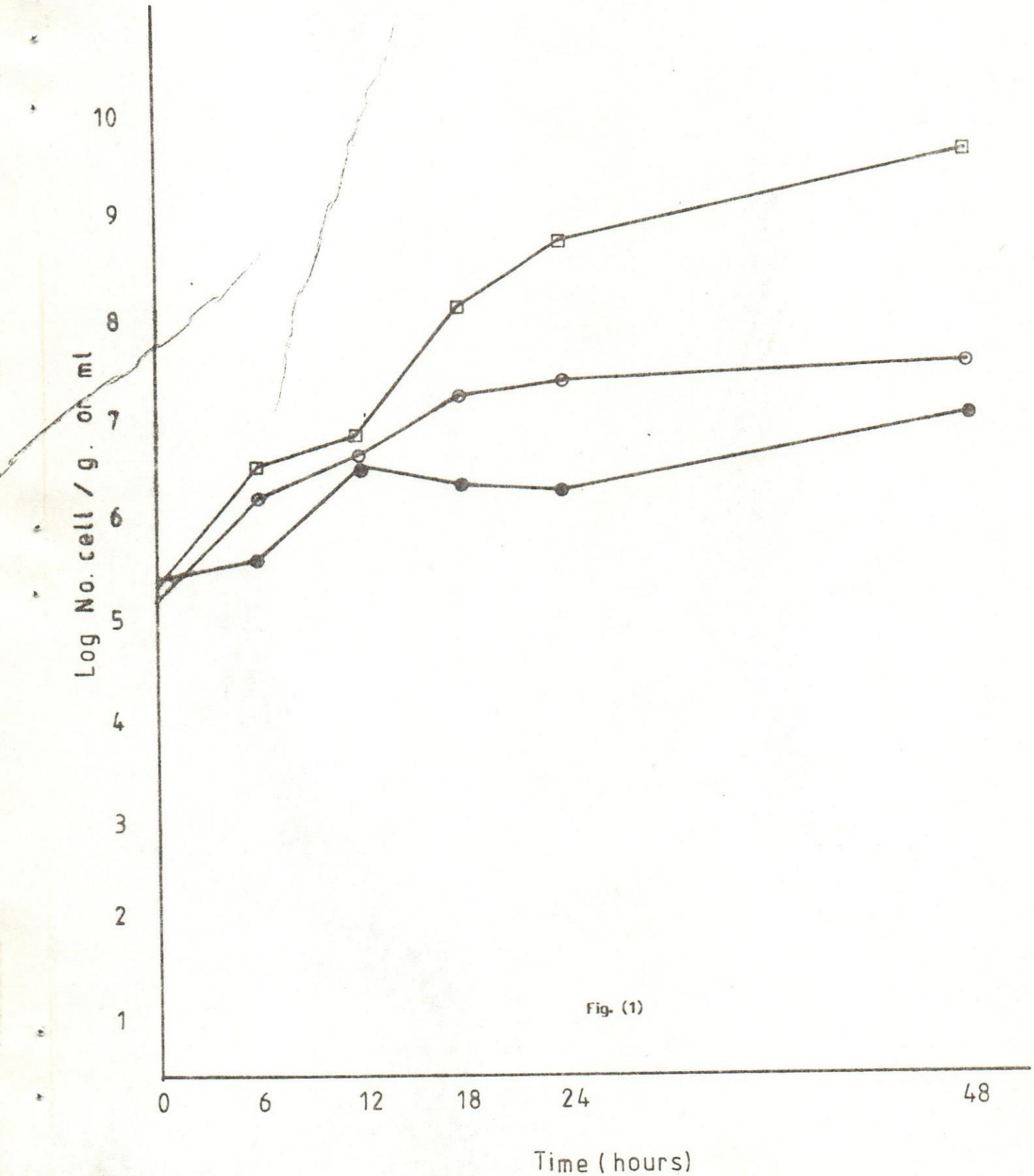

Fig. (1)

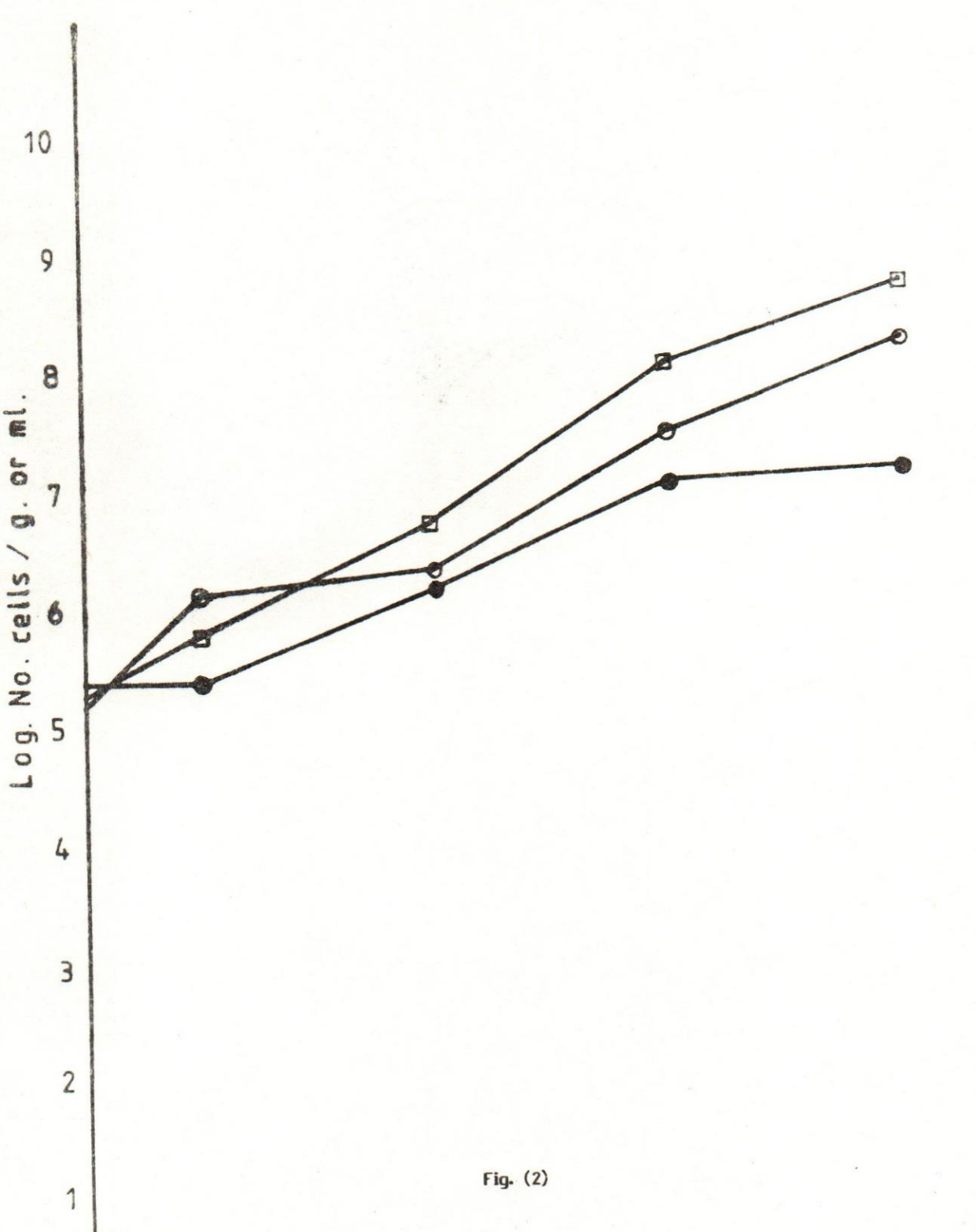

Fig. (2)

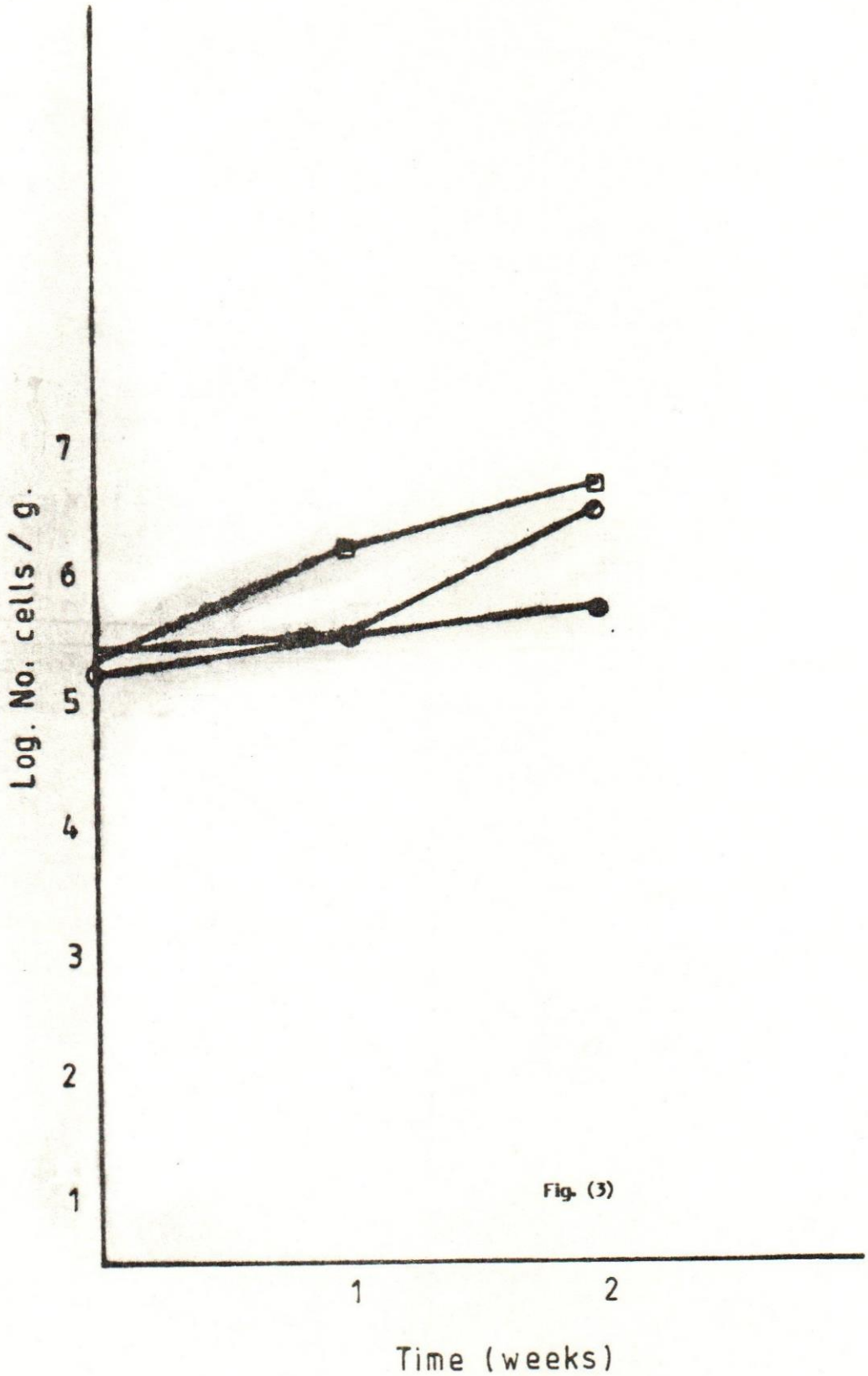

Fig. (3)

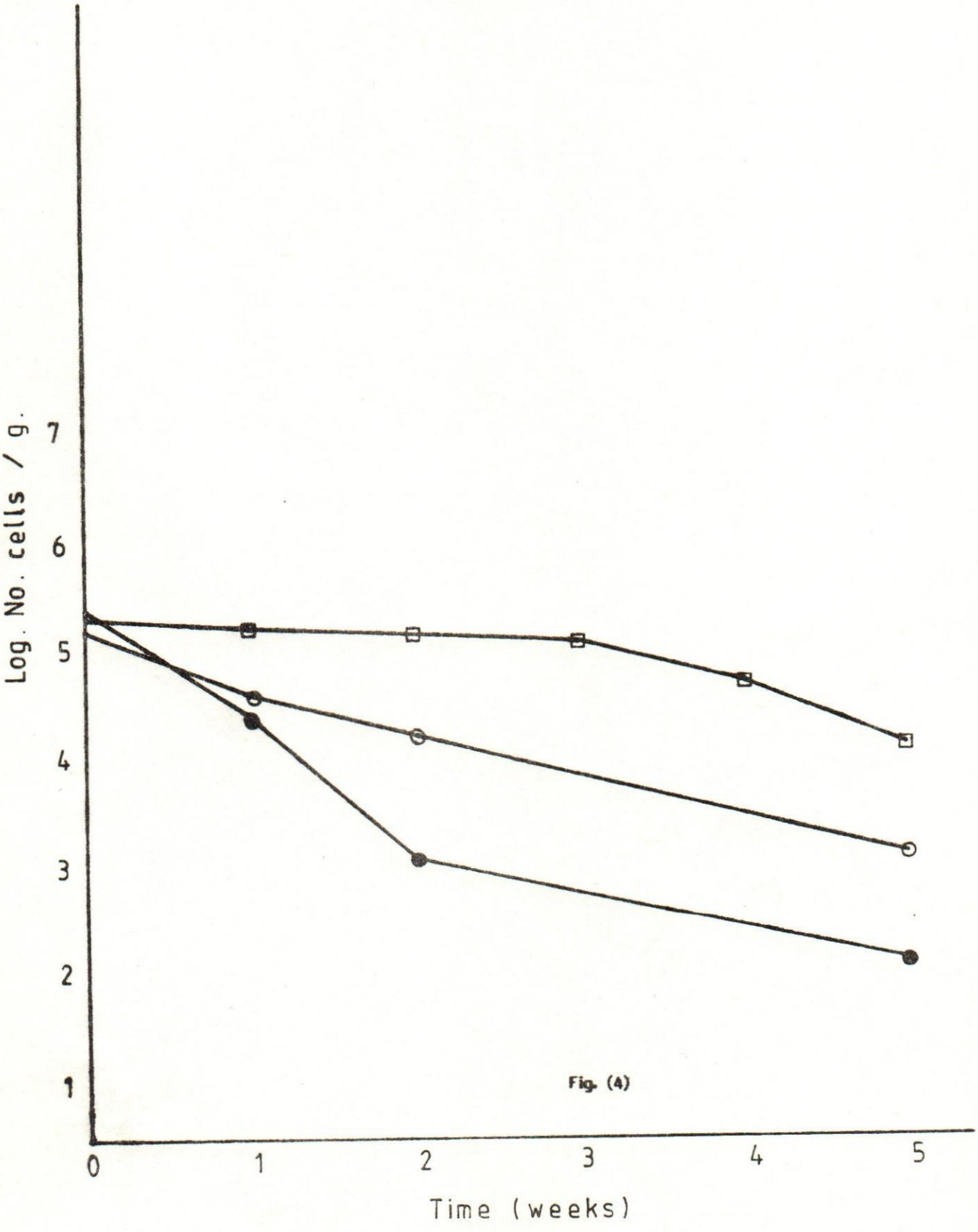

Fig. (4)