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محاولة زرع فيروس النيوكاسل " عترة كوماروف "

في خلايا الانسجـــة (CER)

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أمكن بنجاح زرع فيروس النيوكاسل " عترة كوماروف " في خلايا الانسجة (CER) حتى الامرار الأربعـــون .

وكانت الأعراض الباثولوجية في الخلايا المعدية عبارة عن تغير فـــي شكل الخلايا واستدارتها مصحوبا بموت جميع الخلايا . وعند أقلمـة الفيروس لهذه الخلايا بدأت الأعراض في الظهور بعد ١٨ ساعة من العدوى مع موتها بعد ٣٦ ساعة .

ولقد بدأت قوة الفيروس العيبارية في الزيادة حتى أصبحت ثابتة عند الامرار السادس والثلاثون حيث بلغت ذروتها وهي ($5x10^7$ $TCID_{50}$) في كل سنتيمتر . وعلى هذا فمن المفضل جمع الفيروس عند ٢٠ - ٤٠ ساعة بعد الحقن وهو الوقت الذى تصل فيه القوة العيارية الـــى ذروتها . وعند حقن كتاكيت قابلة للعدوى سن ٤٥ يوما بالامـــرار الأربعون لهذا الفيروس ثم حقنها بالفيروس الضارى بعد ٢١ يومـــا من التحصين وجد أنها اكتسبت مناعة وصدت الفيروس الضارى . ولقـد أعطت أمصال هذه الكتاكيت المحصنة أجساما مناعية من نـــــوع الـ Heamagglutinating قوة ٨٠- ٦٤٠ ، ٣٢٠-١٢٨٠، عند ١٥ ، ٢١ يوما بعد الحقن على التوالـي .

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TRIALS FOR PROPAGATION OF "KOMARO" NEWCASTLE DISEASE VIRUS STRAIN IN TISSUE CULTURE "CER" CELLS

(With 3 Tables & 4 Figs.)

BY
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SUMMARY

Newcastle disease virus ("Komarov" strain) was propagated in CER monolayer cultures for 40 successive passages. Cytopathic changes (CPE) consisted mainly of rounding of the cells followed by complete destruction of the cell sheet. Upon adaptation, CPE started to appear after 18 hours and complete destruction of the cell sheet occured by the end of 36 hours.

The titer started to increase and become constant by the 36th passage reaching its maximum titer (5 x 10 TCID /ml). It is recommended to harvest the virus between the 20th and 40th hour post-infection, the time at which peak titer is obtained.

Fourty five days-old susceptible chickens vaccinated with the 40th passage of this adapted virus, resisted challenge with the virulent virus given 21 days post-vaccination. Immune response in the vaccinated birds demonstrated high HI titers of 80-640 and 320-1280 at 15 and 21 days post-vaccination respectively.

INTRODUCTION

Uptill now vaccines against Newcastle Disease (ND) are prepared in embryonated chicken eggs. The latter are always incriminated as being source of transmitting many pathogens to vaccinated birds (COTTRAL, 1952). Hence many workers tried to propagate ND viral strains in tissue culture cell systems aiming to get an adapted virus without loosing its antigenic properties (BANKOWSKI et al. 1958; HUYGELEN and PEETERMANS, 1963; NAFI, 1963; LARIN, 1964; MARKOVITS and TOTH, 1964; GALE et al. 1965; SOKKAR, 1966; ROSSI and GRISEPPE, 1969; SOKKAR et al. 1969; RUSEV and DILOVSKI, 1971; JULY HIPOLITTO, 1972).

Recently, KASHABAH (1979) succeeded in propagating ND virus "Komarov" strain in bovine kidney as well as in BHK cell cultures. In addition she found that after adaptation, this strain could be used as a vaccine for the immunization of chickens.

The purpose of the present work is to propagate and consequently to adapt "Komarov" strain in CER cells and the possibility of using it as a vaccine against Newcastle disease. Moreover the immune response of chickens vaccinated with this adapted viral strain was studied similar to that used in birds immunized with the classical "Komarov" egg propagated vaccine.

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MATERIAL and METHODS

1. Virus:

A) Vaccine strain: The virus used in this study was the egg adapted mesogenic "Komarov" strain supplied by Weybridge Reference Lab., U.K. in 1970. Its titer was 10 ELD per ml. when titrated in chicken embryos.

B) Challenge virus: A locally isolated field strain (velogenic viscerotropic virus - supplied by Dr. Shibl) was used for this purpose. When titrated in eggs it gave an infectivity titer of 10 ELD per ml.

2. Adaptation of virus in CER cell culture:

CER monolayer cultures were prepared using 199 medium and following the technic of EL-KARAMANY (1979). For propagation and adaptation, the virus ("Komarov" strain) was inoculated into 4 of prescription bottles, 0.2 ml. each.

3. Virus titration:

A) In tissue culture: It was carried out in CER cells following the technic previously described by LENNETTE (1964). The titer was expressed in log TCID per ml., using the formula of Reed and MUENCH (1938).

B) In chicken embryos: Serial ten fold dilutions were prepared and each dilution was inoculated into 5 eggs (9 - 10 days old embryos) by the chorioallantoic route. The embryos were examined daily for a period of a days. Those dieng during the first 24 hours were discarded. Hemagglutination activity as well as ELD were calculated by the formula of Reed and MUENCH (1938).

4. Vaccination of chickens:

Fourty five days old susceptible "Nicols" chickens were supplied by the "General Poultry Organization" as day old chicks. They were kept under strict isolation till being used. Before vaccination their sera were examined by the HI test where they proved to be susceptible to ND virus infection.

The susceptible chickens were divided into three groups. The first two groups consisted each of 16 birds and were inoculated with 10 and 10 ELD of the 40th tissue culture passage. The third group composed of 4 chickens which were left without inoculation as control. At predetermined intervals, seras of vaccinated birds were collected and tested by the HI test for the seroconversion.

Twenty one days post-vaccination, 32 birds were challenged by being intramuscularly (I/M) inoculated, each with 100,000 ELD of the challenge virus. Control birds were similarly inoculated with the same dose of the challenge virus. The challenged birds were kept under observation for 21 days.

RESULTS

1. Cytological changes in CER cells:

For this purpose CER cells were grown on coverships, then infected with the Komarov strain of NDV and examined after being stained with H&E stain for the sequences of cytological changes. In the first five passages characteristic changes appeared after thirty-six hours which

consisted of vacuolation of the cytoplasm, the cells attained larger size than normal, became deeply stained and the nucleus lost its nuclear membrane. Then infected cells became degenerated, leaving empty areas on the glass surface and finally the cell sheet (over 80%) was detached from the glass surface by seventy - two hours post-infection. From the sixth passage till the 15th passage, the process was the same but much rapid starting after 24 hours and be completed after 48 hours. Then from the 16th passage till the 30th passage, the appearance of characteristic cytopathic changes (CPE) was constant being after 18 hours and complete destruction of the sheet occured by the end of 36 hours. One should mention that no inclusion bodies or any other aberrant forms were seen during the process of adaptation (Figure I A).

2. Titration of different passages:

During adaptation, certain passages were chosen and simultaneously titrated in both CER cell culture and chicken embryos. Results of this experiment are presented in Table (1) and Figures (2 & 3).

3. Harvest curve of the 33rd passage of NDV in CER monolayer culture:

At predetermined intervals the total virus output (Released as well as cell-associated) was collected and titrated in CER cell culture, in order to determine the optimum time for harvesting virus to be used in vaccination process. Results of this study are collected in Table (2) and Figure (4).

4. Immunogenic properties of the 40th passage:

Results of the immune response of chicks vaccinated with the tissue culture 40th passage, then challenged 21 days later with the virulent virus, are presented in Table (3).

DISCUSSION

CER cells are a new type of cell culture which proved its effeciency. EL-KARAMANY (1981) and TAHA (1982) succeeded in preparing an inactivated vaccine against Rift Valley fever (RVF) by growing RVF virus in these cells.

In the present work the adaptation of NDV to these cells is clear if one looks to the elapse after which CPE started or be completed. The time for virus adaptation decreased till it become constant, appearing after 18 hours and complete destruction of the cell sheet occured after 36 hours by the 16th - 30th passage. The main cytological changes were rounding of the cells with complete cytolysis by the end of the process. Intracytoplasmic inclusion bodies or syncytial formation were not seen here as mentioned by other workers for NDV with other cell types (DAS and GOLDBERG, 1961; ALEXANDER et al. 1973).

Concerning the influence of the inoculum on the time of appearance of CEP and consequently on the resulting virus titer, preliminary studies revealed that an inoculum of 0.2 ml. per each prescription bottle (i.e. an M.O.I. of 15) gives the best and most constant result.

A comparative titration in eggs and cell culture of the different passages revealed the following points: Fig. (2) demonstrates that the increase of titer was a gradual one. From the first passage the titer starts to increase gradually and the increase between each two successive titrations was ranging between 0.2 log and 1.0 log uptil the 36th passage with the highest between the 15th and 18th passage. From the 36th passage the titer was fixed and there was no increase or decrease for C. five successive passages being 5 x 10 TCID per ml.

Thus it seems that there are two stages. The first one including the first early passages, where the virus has not yet been adapted to these type of cells and as a result, the titer in both tissue culture and eggs was less than that of the original one. This is more evident by having the titer in eggs higher than its respective titer in tissue culture. Thus it may be logic if one considers the titer in eggs and not on tissue culture as the actual titer. The lower tissue culture titers may be attributed to some propabilities, mainly factors in replication cycle (defect in the cycle or less productivity).

The second stage (upto the 21st passage) demonstrates an increase in tissue culture titers but no change in egg titers. This may reflect an increase in the sensitivity of tissue culture without any change in the total output per cells. Mention that at any passage the titer of virus in eggs is always higher than its respective titer in CER passage. Although SEADLE and WINTERFIELD (1956) found higher titers in chick embryo cultures than in eggs, NGUYEN (1970) got low titers in Vero cells than in eggs.

Studeing the multiplication cycle of NDV in CER cell culture, we selected the 33rd passage, since at this level the titer started to be constant, thus considered as being adapted to this type of cells. The peak titer was reached after 21 hours and was maintained for another 21 hours. Therfore it is recommended that the time for best harvest lies between the 21st and 42nd hour post-infection where the maximum titers are seen at these intervals (Fig. 4). Beyond this time, the titer drops and there may be the possibility of thermal inactivation of the virus. CSIKOVARY et al. (1961) obtained peak infective titer between the 20th and 50th hour. On the other hand, NAFAI (1963), got maximum titers between the 12th and the 48th hour in chick embryo fibroblasts. MARKOVITS and TOTH (1964) mentioned high titers after 50 hours and they recommended the virus harvest to be at 48 hours post-inoculation.

Finally one wanted to investigate if this tissue culture adapted virus could be used as a vaccine. For this purpose susceptible chickens were vaccinated with two different doses of the vaccine and then challenged with a fixed dose of virulent virus. Results as presented in Table (3) reveals the immune response of the vaccinated chickens as reflected by high titers of HI antibodies (80 - 1280). Both groups of birds resisted challenge inoculation and its protection was indicated as well by high HI titers. Thus it is important to vaccinate lots of birds with graded amounts of virus vaccine to determine the least protective dose. In addition to study the duration of this acquired immunity.

In conclusion by propagating NDV in CER cells, we reached a suitable titer, somewhat comparable to the egg titers, which validates its use as an efficient vaccine replacing the classical egg propagated virus. By this way one could avoid the possibility of contamination (latent microbial egg infections) which was the permanent wory many workers.

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Table (1)

Titers of NDV tissue culture passages in both CER cell culture and 9 days old chicken embryos

No of tissue culture passage	Titer in tissue culture expressed in $\log_{10} TCID_{50}$ per ml.	Titer in chicken embryos expressed in $\log_{10} ELD_{50}$ per ml.
3	3.2	6.6
6	3.7	7.5
9	4.5	6.5
12	4.7	6.4
15	5.2	7.5
18	6.5	6.5
21	6.7	6.1
24	7.2	6.2
27	7.2	N.D.
30	7.5	7.8
33	7.5	7.8
36	7.7	8.3

ND = not done.

Table (2)

Virus growth of the 33rd passage of NDV in CER cells.

Time in hours	Titer of harvest expressed in $\log_{10} TCID_{50}$/ml.
3	3.3
6	4.3
9	4.3
12	5.3
15	5.8
18	5.8
21	6.3
24	6.3
27	6.3
30	6.3
33	6.0
36	6.3
39	6.3
42	6.3
45	5.7

Table (3)
Immune response of chicks vaccinated with the tissue culture 40th passage of NDV

Table (3) : Immune response of chicks vaccinated with the tissue culture 40th passage of NDV.

Type of vaccine	Dose of virus vaccine	HI titers at the following days post-vaccination			Deaths post-challenge
		0	15	21	
Tissue culture	10^5 ELD_{50}	less than 20	560* (320 - 640)	880* (320 -1280)	0/16
	10^6 ELD_{50}	" " "	190* (80 - 320)	1070* (640-1280)	0/16
Egg adapted	10^5 ELD_{50}	" " "	160* (80 - 320)	360* (160-640)	0/4
	10^6 ELD_{50}	" " "	240* (80 - 640)	400* (80 -1280)	0/4
Control	---	" " "	---	---	4/4**

* Average of the group .

** Birds died with ND symptoms on the 4th - 7th day post - challenge.

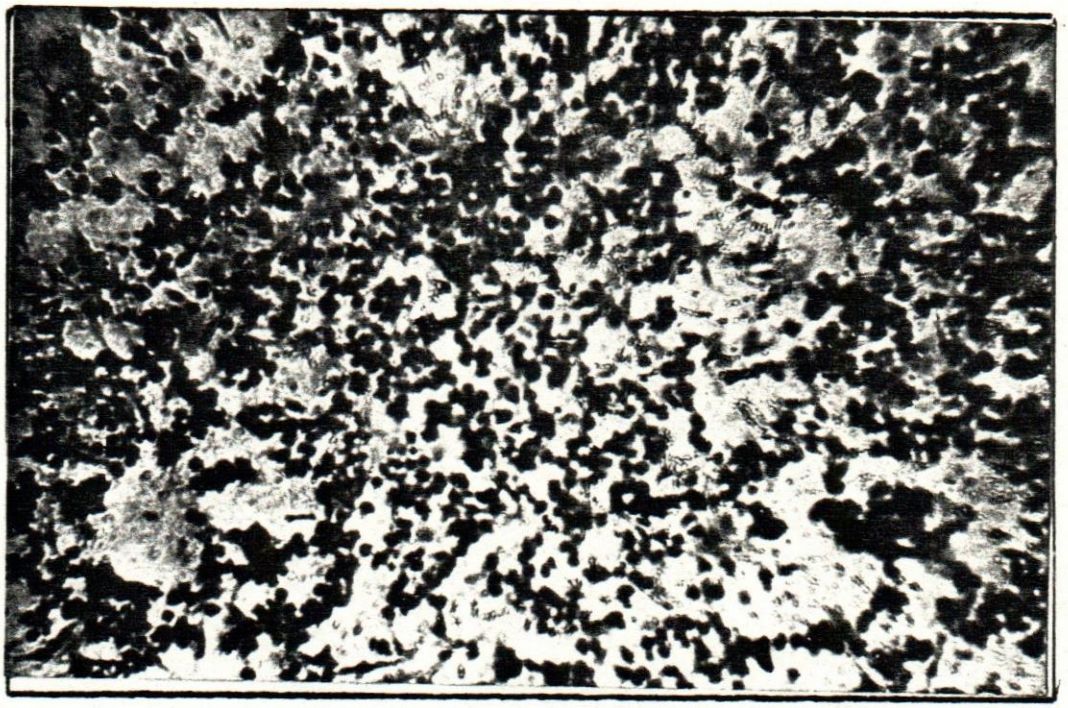

Fig. (1 a): CER cells infected with ND virus (x 40).

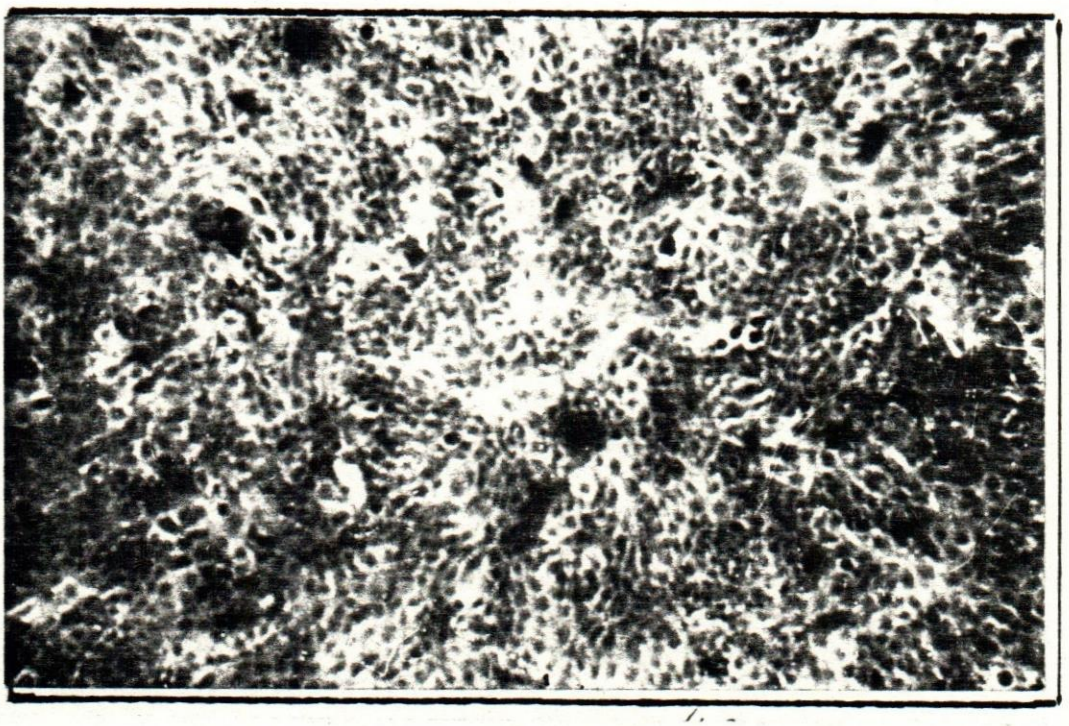

Fig. (1 b): Normal CER cells (x 40).

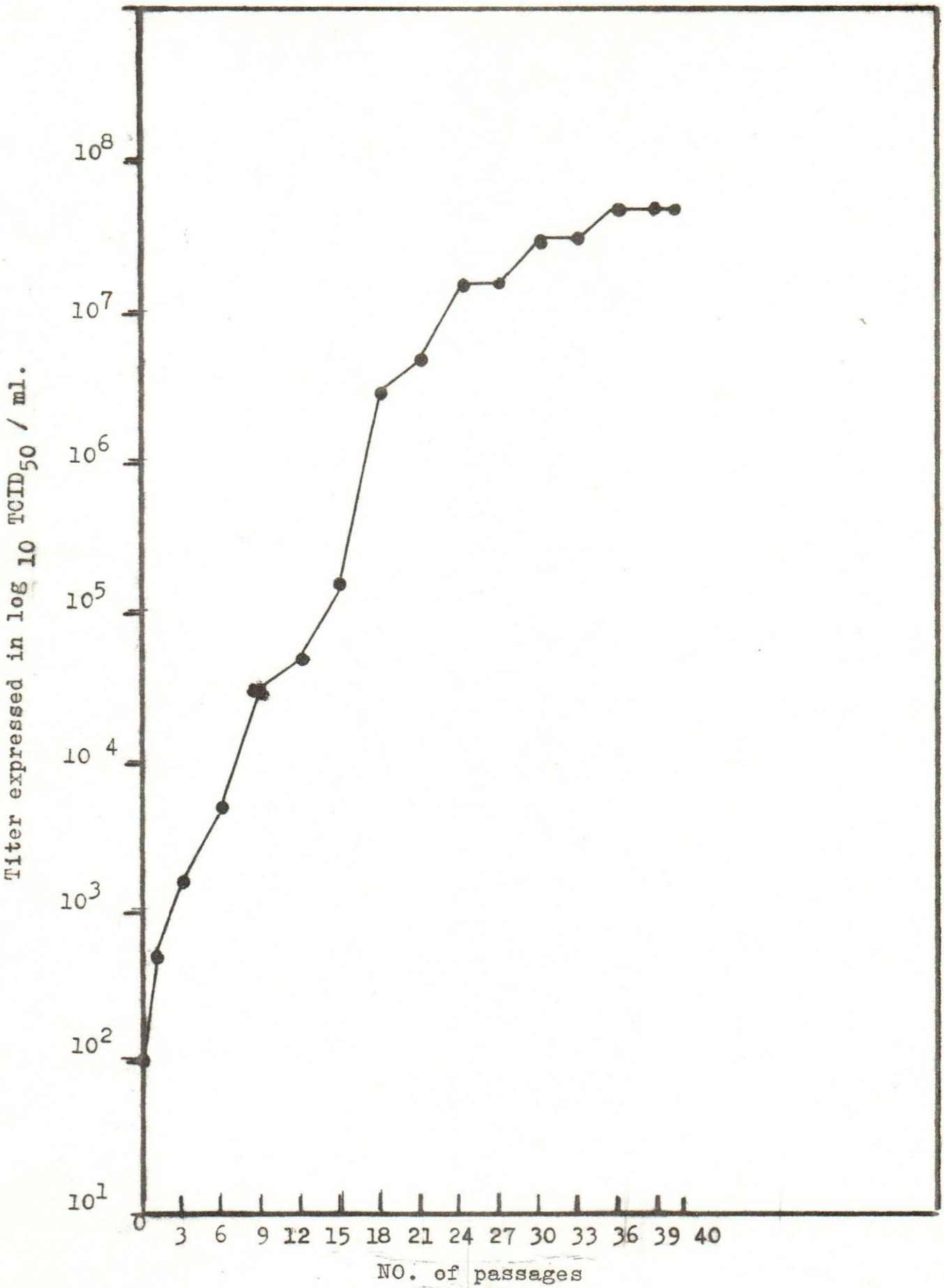

Figure (2) : Titers of different passages of NDV in CER cell culture.

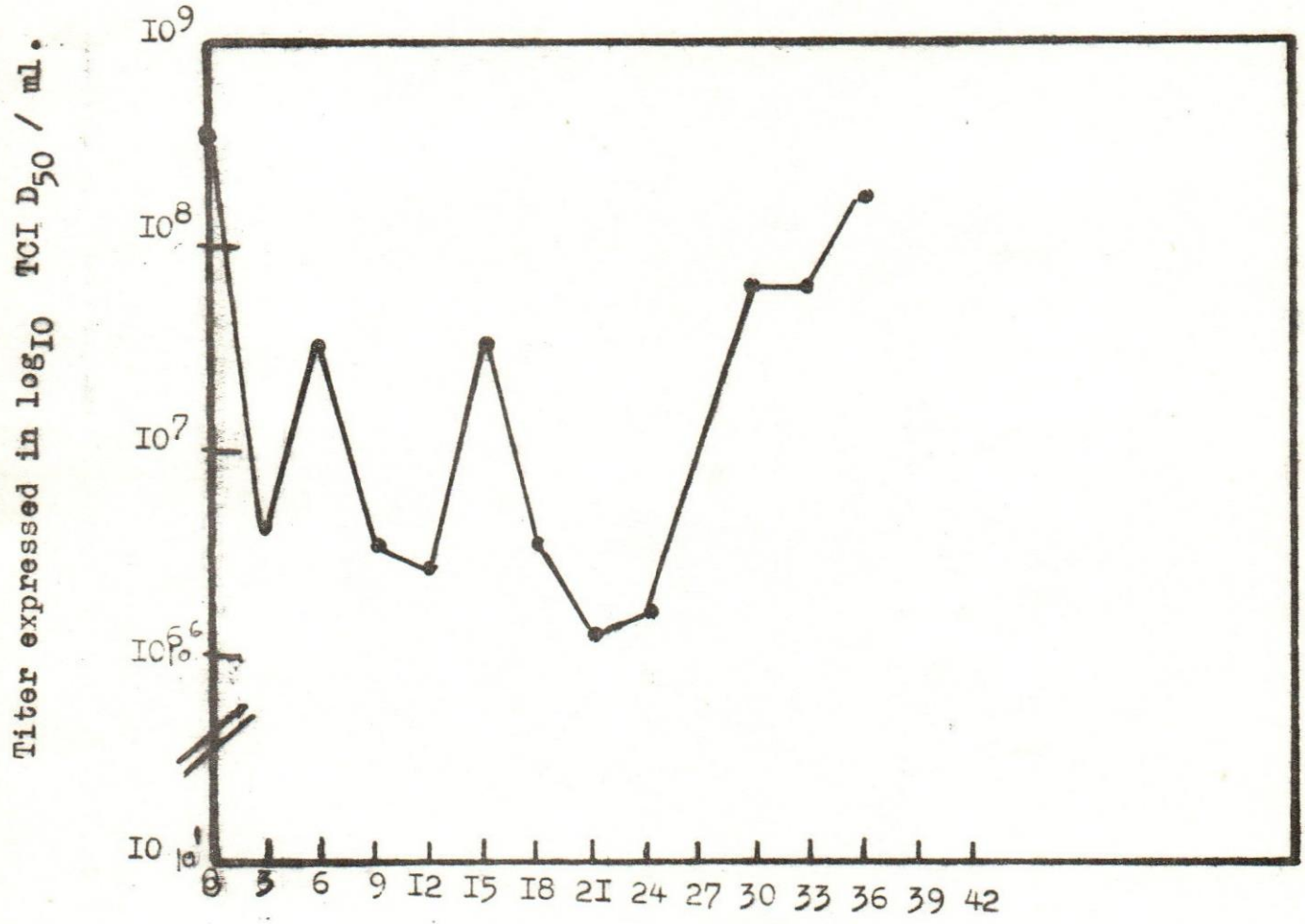

Figure (3) : Titers of successive Newcastle disease virus tissue culture passages in 9 days old chicken embryos .

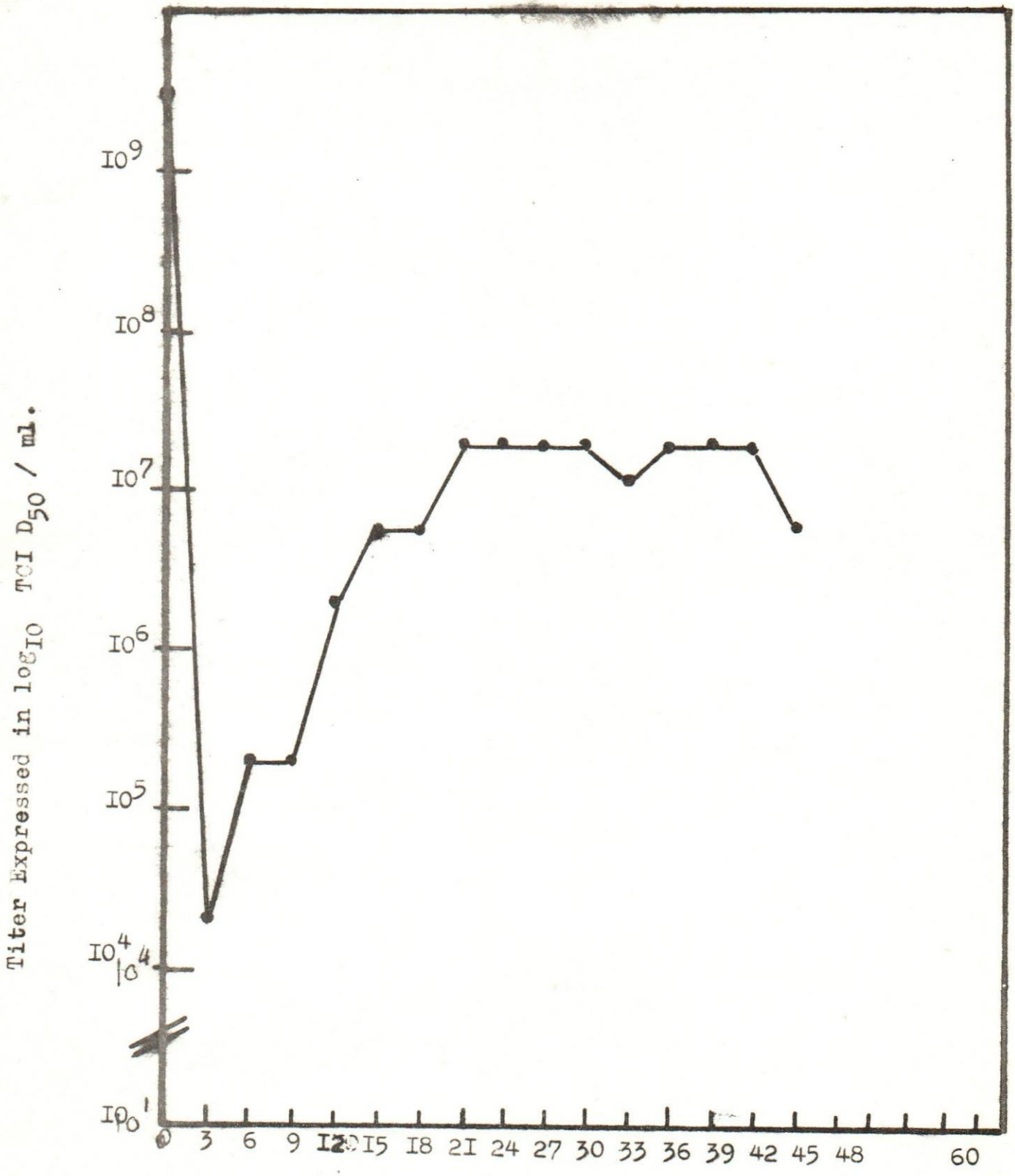

Figure (4) : Harvest Culture of the 33 rd NDV passage in CEP Cells .