

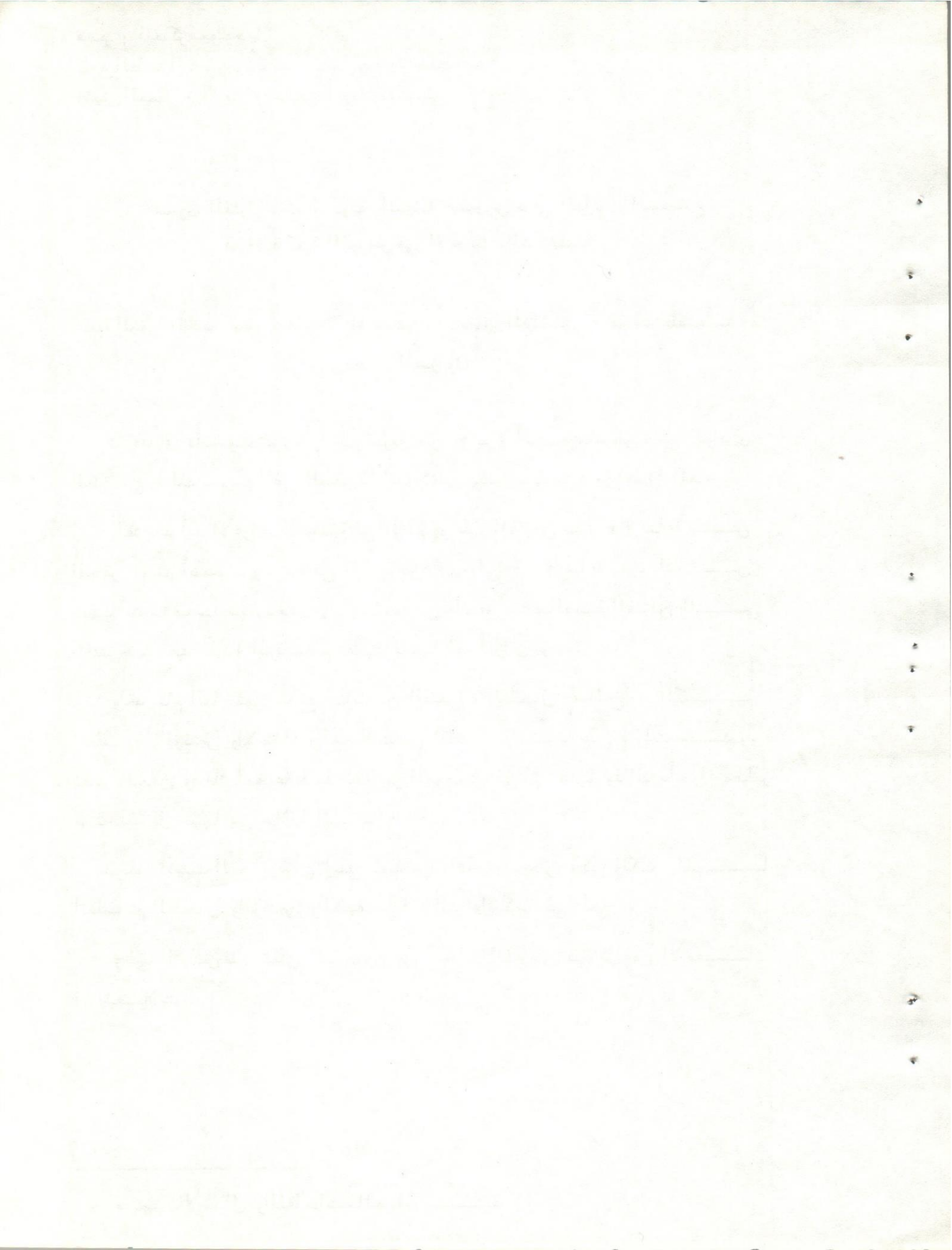
عدوى الفئران السويسرية البيضاء بفيروس حمى الوادى المتصدع  
دراسة كمية الفيروس فى الأحشاء الداخلية

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تم عدوى مائة وثلاثون فأرا سويسريا سن ٣ - ٤ أسابيع بفيروس حمى الوادى المتصدع وذلك بحقنها فى التجويف البريتونى بكمية محددة من هذا الفيروس ولقد بدأت الأعراض المرضية فى الظهور على الفئران بعد ٢ ساعة من الحقن ، ثم أصبحت واضحة فى المدة من ٤٥ الى ١٠٢ ساعة بعد الحقن ثم بدأت تخف حدتها بعد ١١٤ ساعة من الحقن ، وبالنسبة للفئران التى ظلت حية بعد هذا الوقت فلم تظهر عليها أية أعراض مرضية .

ولقد قام الباحثون بجمع عينات من الكبد ، الطحال ، المخ ، الكلية القلب ، والرئتين والأمعاء والعضلات من الفئران المعدية على فترات محددة بعد الحقن وذلك لمعرفة كمية الفيروس الموجودة فى كل منها وذلك بأجراء معايرة مستحلب كل منها فى خلايا الـ CER

ولقد أثبتت المعايرة أن أعلى كمية من الفيروس موجودة فى الكبد يليها القلب ثم الطحال والرئتين والكلية بينما كانت أقل كمية فى المخ . وعلى العكس فلم يتمكن الباحثون من أيجاد الفيروس فى كل من الأمعاء أو العضلات .



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**PATHOGENESIS OF RIFT VALLEY FEVER VIRUS  
(RVFv) IN SWISS-ALBINO MICE :**  
**VIROLOGICAL ASSAY OF THE VIRUS CONTENT IN INTERNAL ORGANS**  
(With 2 Tables and 5 Figures)

By

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**SUMMARY**

One hundred and thirty swiss-albino mice of 3-4 weeks old were each inoculated intraperitoneally (1/p) with a fixed dose of RVF virus.

The first symptoms started to appear after 42 hours. The course of the disease progressed and the symptoms became apparent between 54 to 102 hours, then started to decline by 114 hours. Mice surviving at the end of the experiment did not manifest any clinical symptoms.

Livers, spleens, brains, kidneys, hearts, lungs, intestines and skeletal muscles were collected at predetermined intervals for quantitative assay of the virus content in the various organs. Virus content as detected by titration in CER cell cultures was higher in the liver than the heart, spleen, lung, kidney and less in the brain. Yet, virus could not be isolated from the intestines and skeletal muscles of infected mice.

**INTRODUCTION**

Rift valley fever virus (RVFv.) infects sheep, cattle and man (DAUBNEY and HUDSON, 1932) as well as species of monkeys (FINDLAY, 1932) and some species of rodents (Mc INTOCH, 1961). However, the susceptibility of other animals to natural or experimental infection with the virus is apt to many conditions and factors. In addition, a country may be infected for a time with RVFv, then the disease disappears and reappears once again.

It has been claimed that rodents may act as reservoirs of the virus without showing apparent symptoms (KASAHARA and KOYAMA, 1973). During the last outbreak of RVF infection in Egypt, MEEGAN and MOUSSA (1978) tried to detect the virus in the sera of rodents. EL-SANOUSI (1979) detected antibodies in the sera of wild rats in Egypt, but no virus could be detected.

Therefore, it was important to investigate the course of the disease and the pathogenesis of RVFv in swiss-albino mice, since these laboratory animals are widely used in virological field for many purposes such as : isolation, propagation of the virus and evaluation of the vaccine (SMITHBURN, 1949; KITCHEN, 1950 and BENNETT *et al.*, 1965).

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The purpose of the present work is to explore the pathogenesis of RVF virus in experimentally infected mice, by quantitative estimation of the virus in various organs from dead and/or mice sacrificed at predetermined intervals post-inoculation with a constant amount of RVFv. This may be of value in elucidating the role played by this species in the epidemiology of the disease and their role as virus carriers.

## MATERIAL and METHODS

### Material :

1. **Virus** : RVF virus locally isolated in Egypt was used in this study. It had a titer of  $10^7$  MIPLD<sub>50</sub> per ml. when titrated in adult mice.
2. **Cell culture** : CER cells obtained from Wistar Institute, Philadelphia, U.S.A., were propagated and maintained in the laboratory as described by TAHA (1982).
3. **Animals** : 3-4 weeks Swiss-albino mice were used for this purpose.

### Methods :

1. **Virus isolation** : It was done in CER cells following the techniques previously described by EL-NIMR (1980) and TAHA (1982).
2. **Virus titration** : Quantitative assay of the virus content in various organs was done according to the methods described by EL-NIMR (1980) and TAHA (1982). The titer was expressed as  $\log_{10}$  TCID<sub>50</sub> per ml of the original inoculum using the formula of REED and MUENCH (1938).

### Experimental studies :

One hundred and thirty mice were each inoculated intraperitoneally (i/p) with 0.2 ml. of RVF virus containing C.5000 MIPLD. In addition, forty mice were left without inoculation as controls. Parts from livers, brains, spleens, kidneys, lungs, hearts, intestines and skeletal muscles were collected from infected and control mice at predetermined intervals. The first samples were collected after 6 hours post-inoculation, then the samples were collected every 12 hours till the 6<sup>th</sup> day and then every 24 hours till the 15<sup>th</sup> day post virus-inoculation.

## RESULTS

### 1. Clinical symptoms :

The first signs of illness appeared after 42 hours post-inoculation where the mice showed some nervous symptoms such as restlessness and running aimlessly. At 54 hours post-infection, the symptoms became more intense being paralysis of the hind limbs, hunched position, dilatation of the eye ball, sudden respiratory movements, loss of appetite and the mice are away from each other which end by death. Then, the symptoms started to subside till ending after 150 hours where no clinical symptoms were found. Tale (1) illustrates the number of dead mice per interval.

### 2. Virological assay :

Quantitative assays of the virus in the various organs of mice sacrificed and/or died at various intervals post-virus infection are presented in Figures (1, 2, 3, 4 & 5) and Table (2).

## RIFT VALLEY FEVER IN MICE

Results revealed that the virus could be isolated from the livers, spleens, lungs, brains and kidneys of infected mice but not from the skeletal muscles and intestines. In a descending order the highest titer was obtained from the liver, then heart, spleen, lung, kidney and brain. Yet, the highest isolation rate was from liver followed by lung, brain, spleen kidney and heart.

## DISCUSSION

Mice inoculated with RVFv. (c. 5000 MIPLD<sub>50</sub>/0.2 ml.) manifested clinical signs which are characteristic of RVFv infection (MIMS, 1956 a). The symptoms appeared within the period from 42 hours to 6 days and could be classified into three phases: the first from 42-54 hours, with a death rate of 8%. This phase is an early phase since it agreed with the rate of isolation of the virus from various organs especially the liver. The second phase started from 66 hours till 102 hours post-infection with a death rate of 42%. The third phase was seen by 102 hours where the symptoms started to decline and the death rate reached only 24.3%. The actual death rate in the present study was about 74.3%. Generally the real course of the disease in albino mice showing characteristic symptoms was of 6 days, although mice die after this interval may not show symptoms but still had the virus in their bodies. MIMS (1956 c) stated that an occasional death after 8-10 days is shown to be due to infection resulting from cannibalism. It seems that this does not apply in the present study since the number of dead mice after 8-10 days is very small.

Results of isolating RVFv from the various organs as presented in table (2) showed that the titers of the liver are the highest ones (average  $10^{4.75}$  TCID<sub>50</sub>/ml) and those of the brain the lowest (average  $10^{3.25}$  TCID<sub>50</sub>/ml.). The virus content in the livers of mice sacrificed and/or died at various intervals postinfection is represented in Fig. (1). Three phases could be demonstrated: the first between 6 till 54 hours with no increase in virus titer; the second starts after 54 hours and it is the time of increase in titer with the peak by the 102<sup>nd</sup> hours ( $10^{6.5}$  TCID<sub>50</sub>/ml.), the third begins after 126 hours till the end of the experiment with fluctuations in titer ( $4.5-3.5$  TCID<sub>50</sub>/ml.) but always below the peak of the second phase.

With respect to the virus content in the spleen, it is clear from Fig. (2) that the first isolation was after 30 hours post-infection, then the titer rises reaching the first peak at 66 hours ( $10^{6.0}$  TCID<sub>50</sub>/ml.). The second peak was after 138 hours ( $10^{6.0}$  TCID<sub>50</sub>/ml.), then a rapid decrease in titer occurs till the end of the experiment ( $10^{3.0}$  TCID<sub>50</sub>/ml.).

Virus content in the lungs runs a course parallel to that of the spleen (Fig. 3). This means a rise in titer with a peak at 138 and 150 hours with a gradual drop after this interval till reaching  $10^{2.5}$  TCID<sub>50</sub>/ml. by the end of the experiment. Concerning the brain, Fig. (4) illustrates three phases. The first between the 30<sup>th</sup> and 66<sup>th</sup> hours where the titer was nearly constant ( $10^{4.0}$  TCID<sub>50</sub>/ml). Then a second phase occurs by 78<sup>th</sup> hours with the peak reached after 114 hours ( $10^{4.5}$  TCID<sub>50</sub>/ml) and finally a third phase which starts from the 126<sup>th</sup> hours till the end of the experiment with a decrease in titer.

RVF virus could be isolated from the kidneys after 42 hours, followed by an increase in virus content with the peak after 78 hours ( $10^{5.2}$  TCID<sub>50</sub>/ml.). This peak titer was followed by decline in titer till reaching  $10^{2.0}$  TCID<sub>50</sub>/ml. by the end of the experiment.

No virus could be detected in skeletal muscles or intestines which means that no multiplication occurred in these sites.

One can postulate that adult mice are more susceptible to RVFv infection through the intraperitoneal route (i/p). The liver being the nearest organ, so the virus can reach it

after 1/p inoculation. The data permit the conclusion that the liver was a primary site of multiplication (DAUBNEY and HUDSON, 1932; FINDLAY, 1933; EASTERDAY *et al.*, 1962 and TOMORI and KASALI, 1979). In addition, the strain used in this work is a hepatotropic one being adapted as a pantropic virus more than a neurotropic one which needs more successive passages by the intracerebral route (SMITHBURN, 1949). This explains the low titer of the virus content of the brain.

Thus one can conclude that the liver is a target organ for RVE virus being the one of choice for virus multiplication and then dissemination to other organs occurs subsequently.

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## RIFT VALLEY FEVER IN MICE

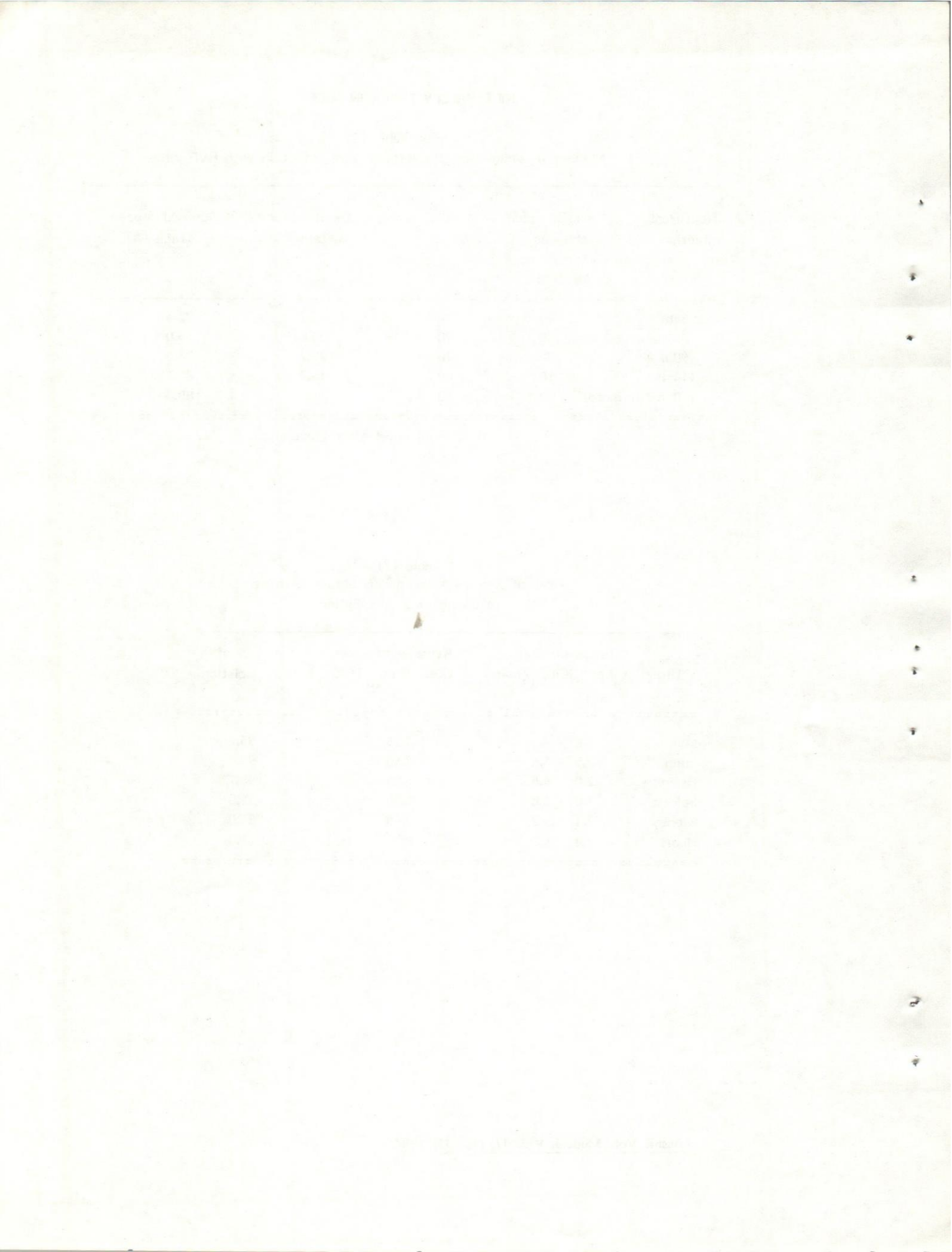
**Table (1)**  
Number of dead mice per interval post-infection with RVF virus

Time Post infection	No of mice showing symptoms or died	No of surviving mice	Death percentage (%)	Survival percentage (%)
54h.	10	120	7.7	92.3
66 h.	30	90	25.0	75.0
90h.	20	70	22.2	77.8
114 h.	10	60	14.3	85.7
150 h (or more)	7.7	53	11.7	88.3

h = hours post-virus inoculation.

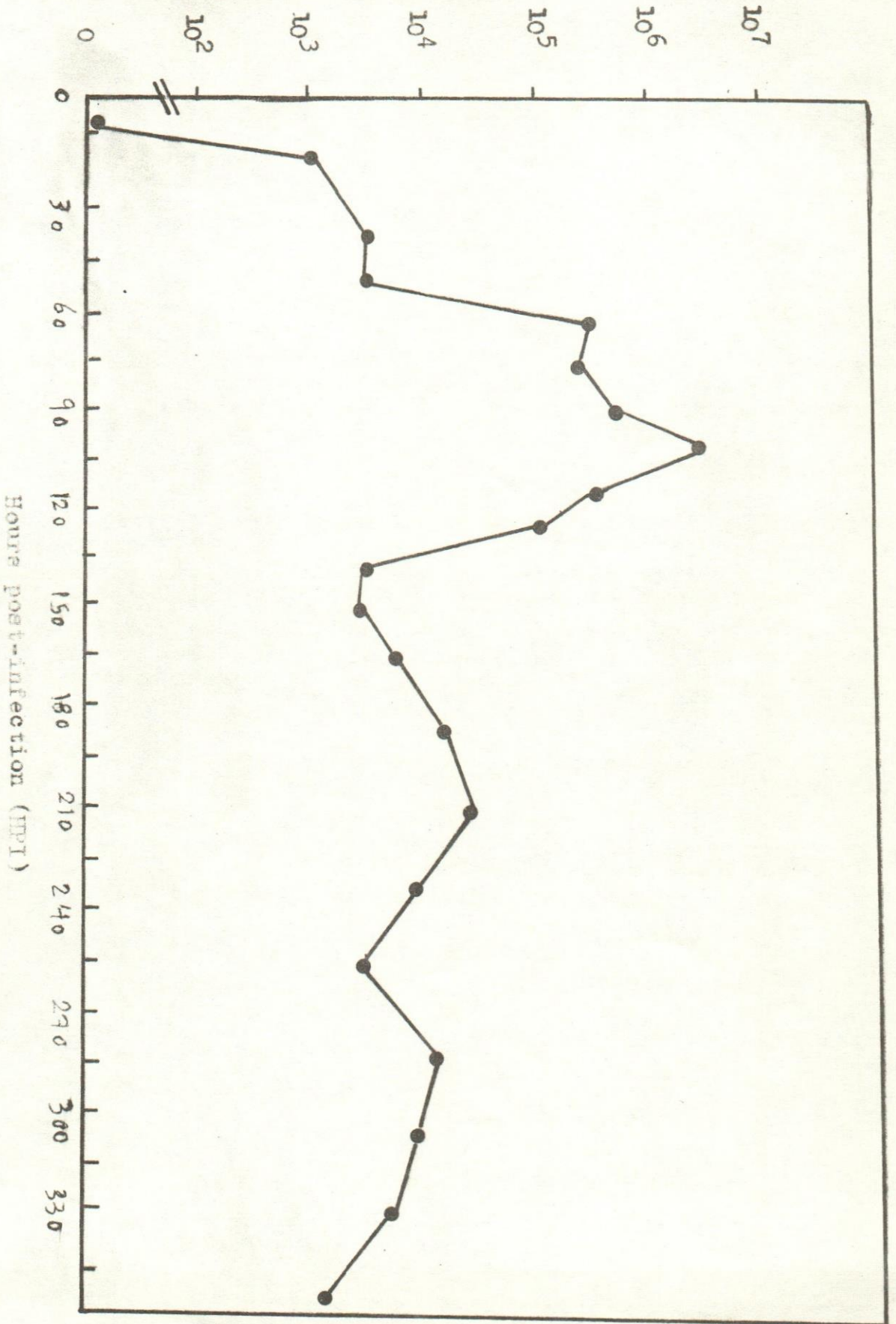
**Table (2)**  
Titer of Rve virus in various organs of mice following virus inoculation

Organ	Range of titer (Log <sub>10</sub> TCID <sub>50</sub> /ml.)	Average of titers (Log <sub>10</sub> TCID <sub>50</sub> )	% of isolation
Liver	3.0 - 6.5	4.75	95.2
Lung	2.5 - 5.6	4.50	95.0
Brain	2.0 - 4.5	3.25	94.7
Spleen	3.0 - 6.0	4.50	91.0
Kidney	2.1 - 5.2	3.65	85.0
Heart	3.4 - 6.5	4.75	71.0

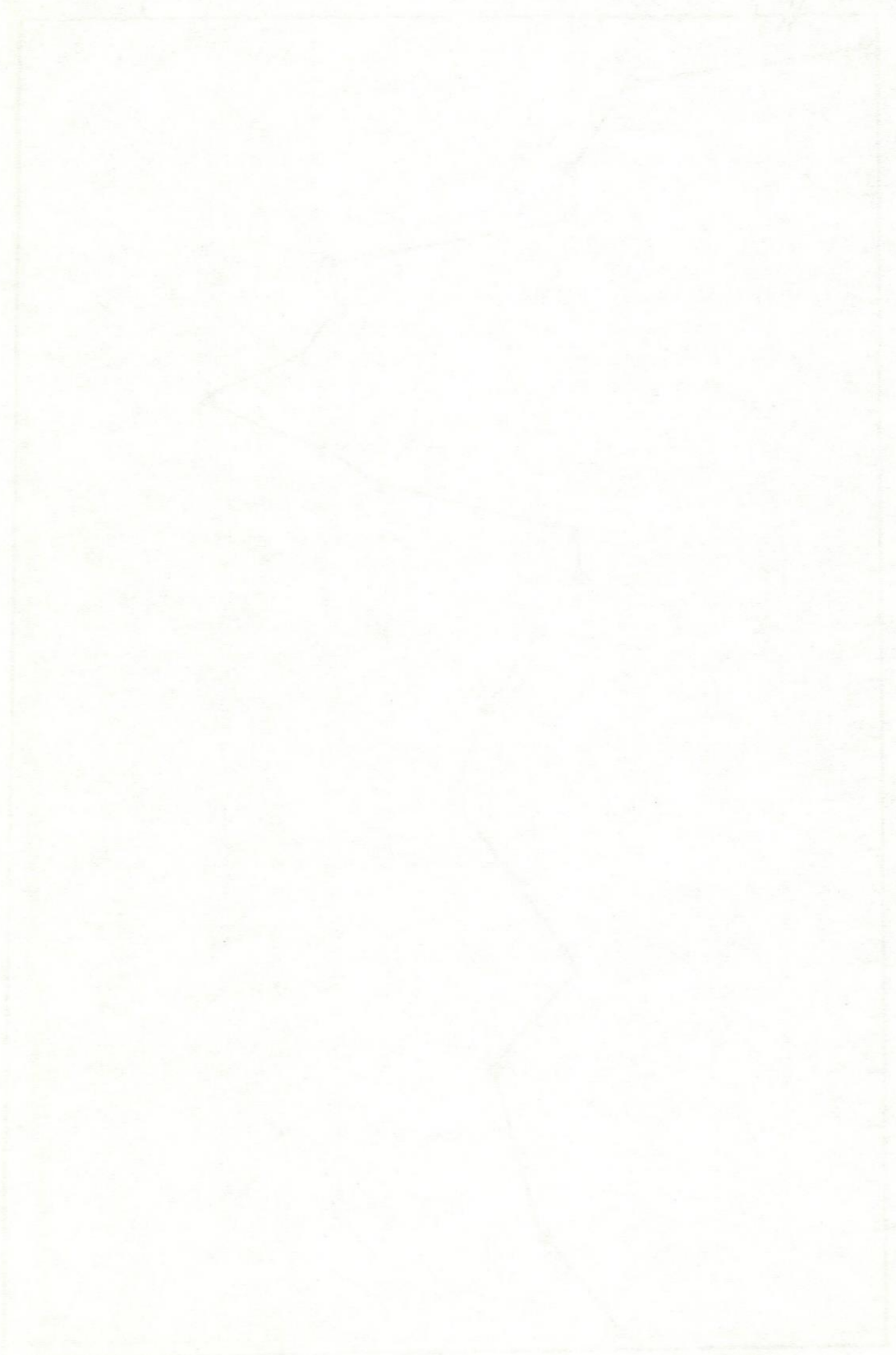




Titer expressed in log TCID<sub>50</sub>/ml.

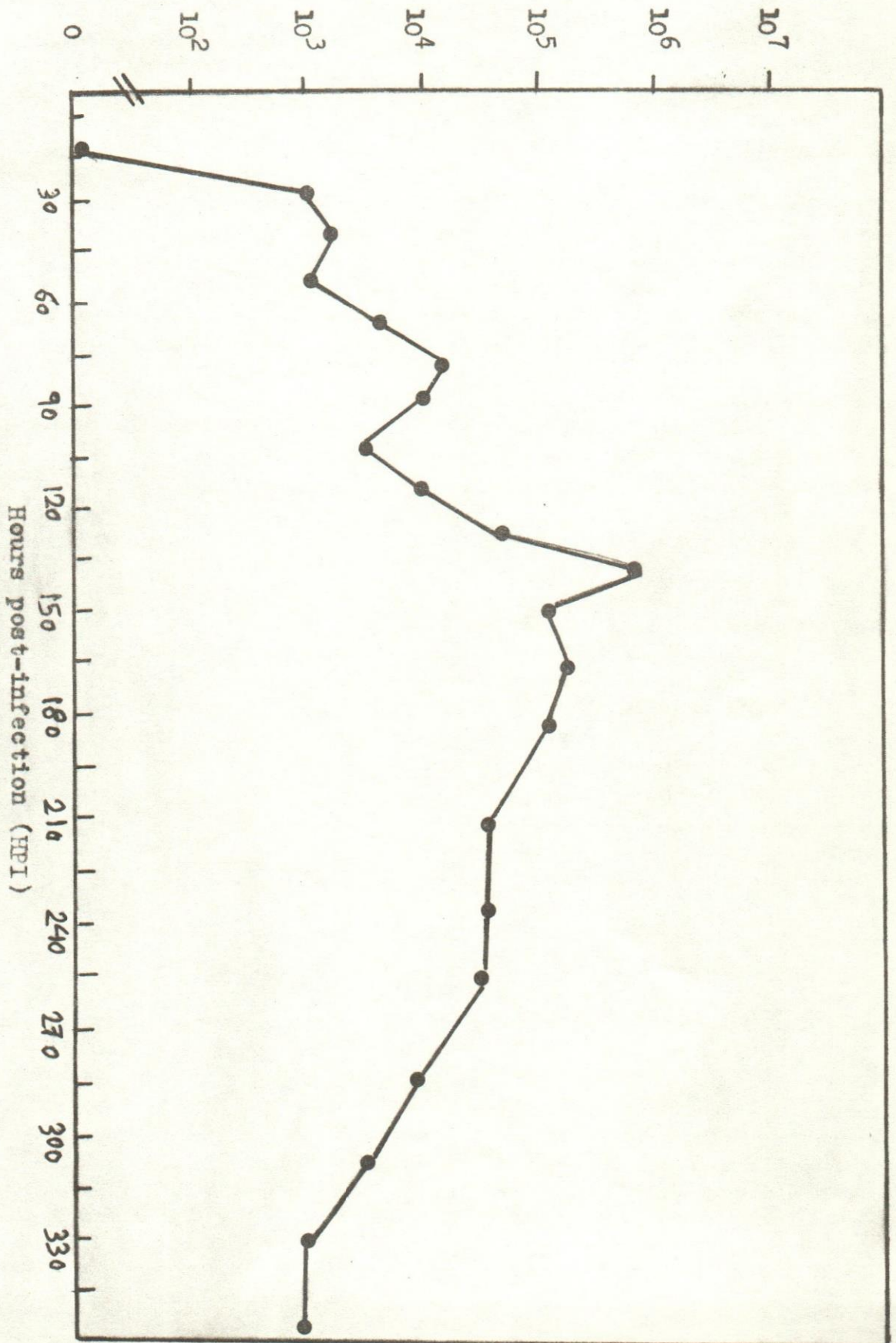


Figure(1): Titers of RVF virus in livers of mice collected at various intervals post-inoculation.

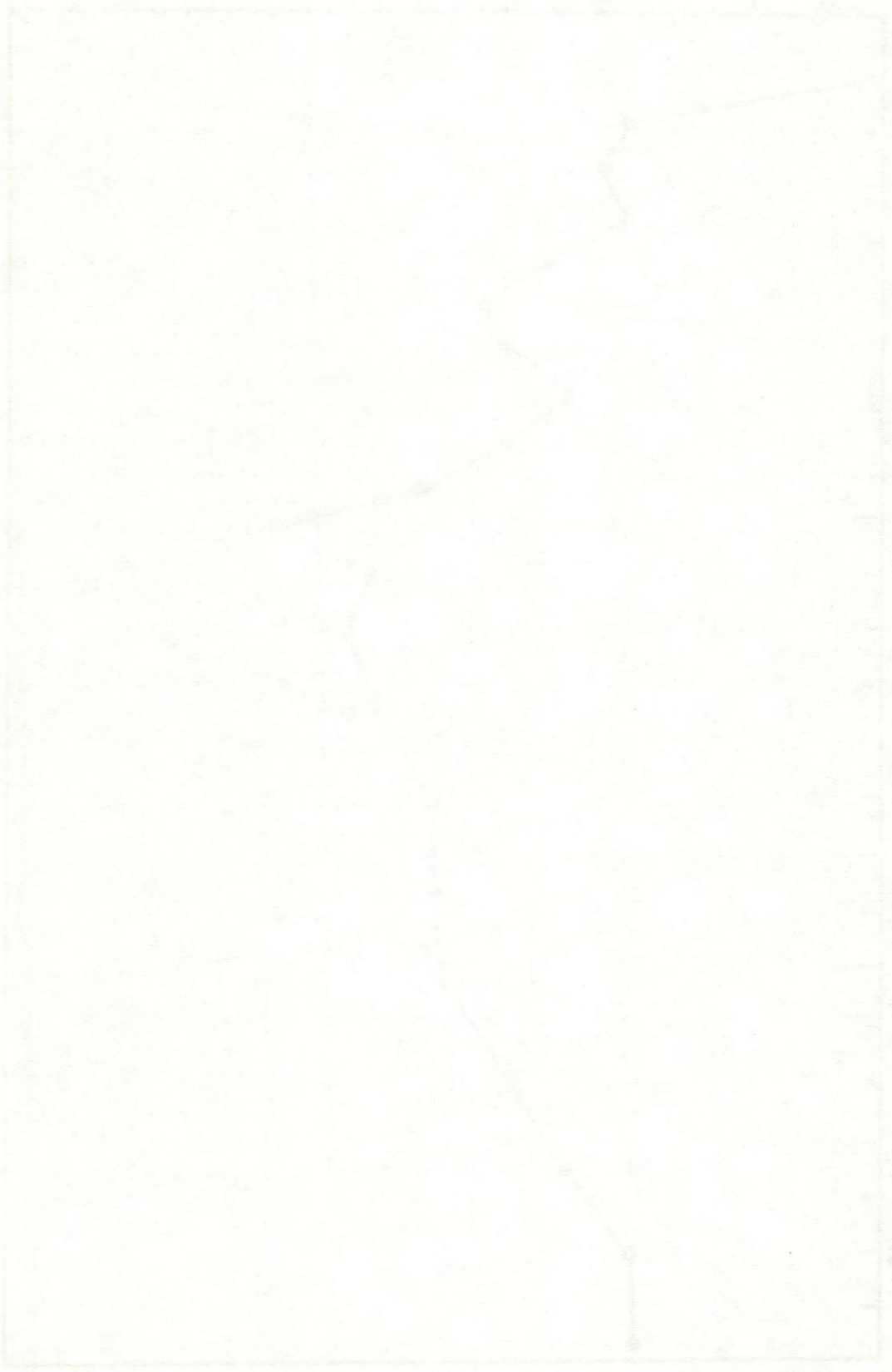


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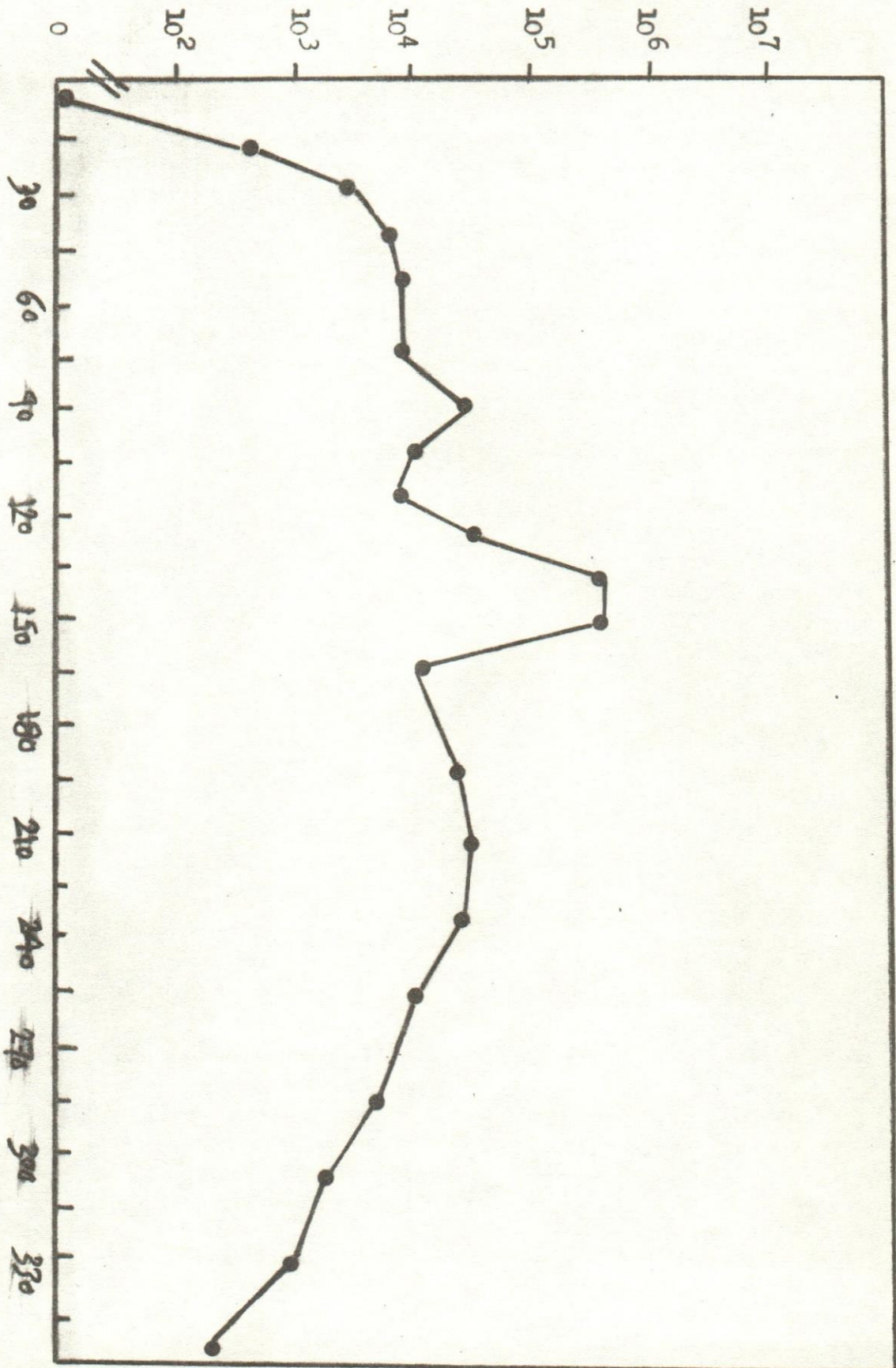


Figure(2): Titers of RVP virus in spleens of mice collected at various intervals post-inoculation.

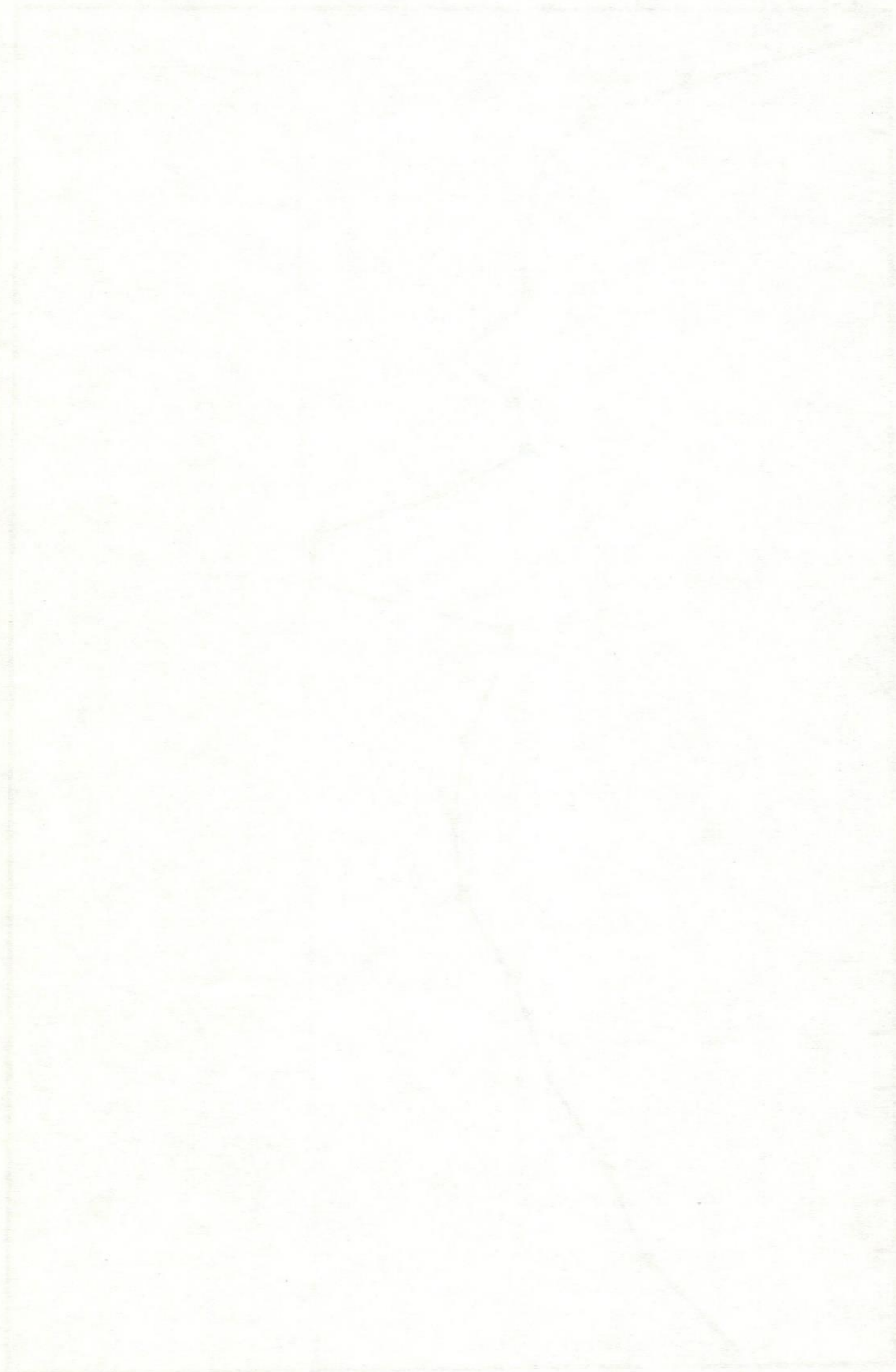


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Titer expressed in log TCID<sub>50</sub>/ml.



Figure(3): Titers of RVF virus in lungs of mice collected at various intervals post-inoculation.



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Titer expressed in log TCID<sub>50</sub>/ml.

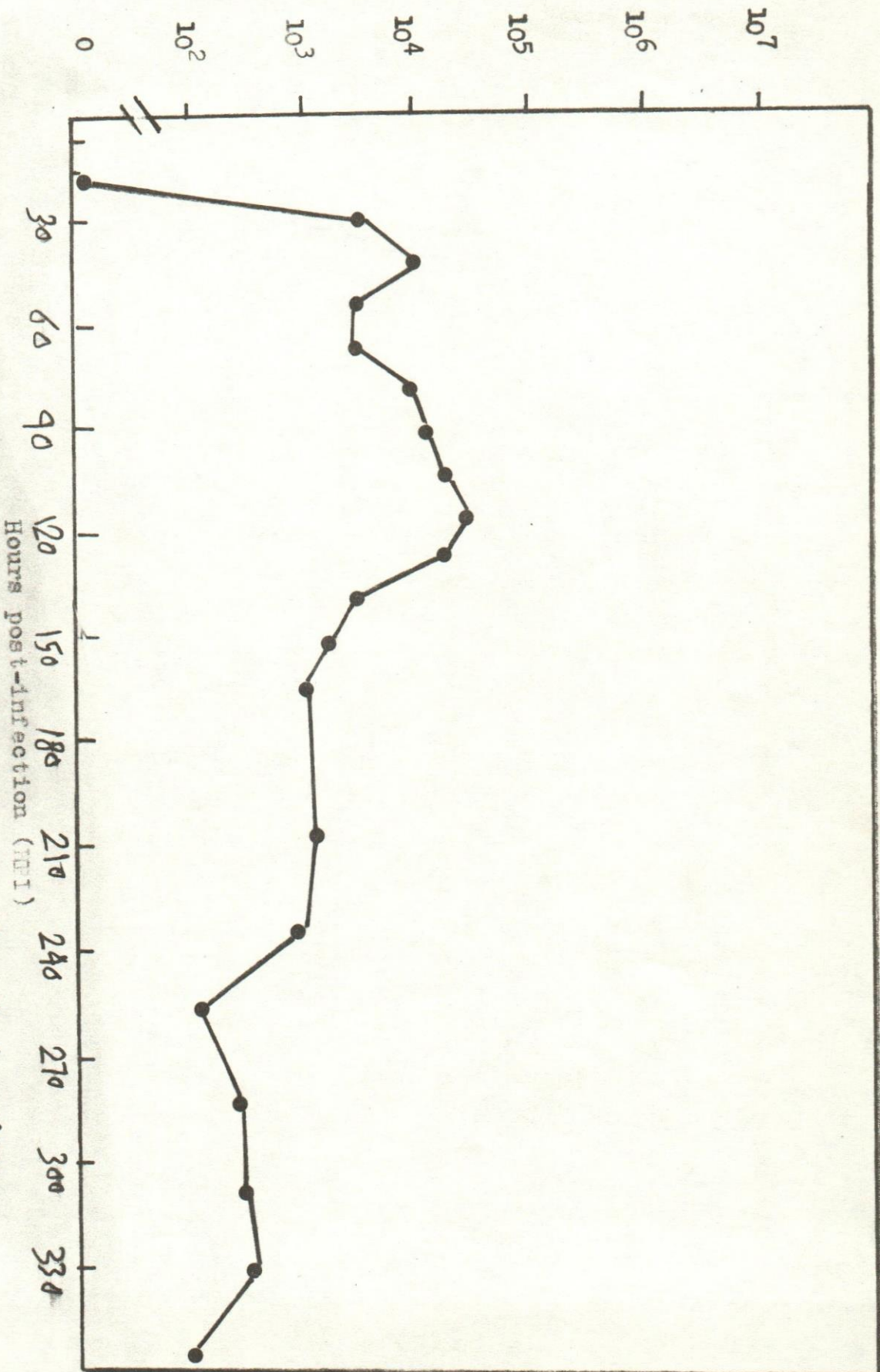
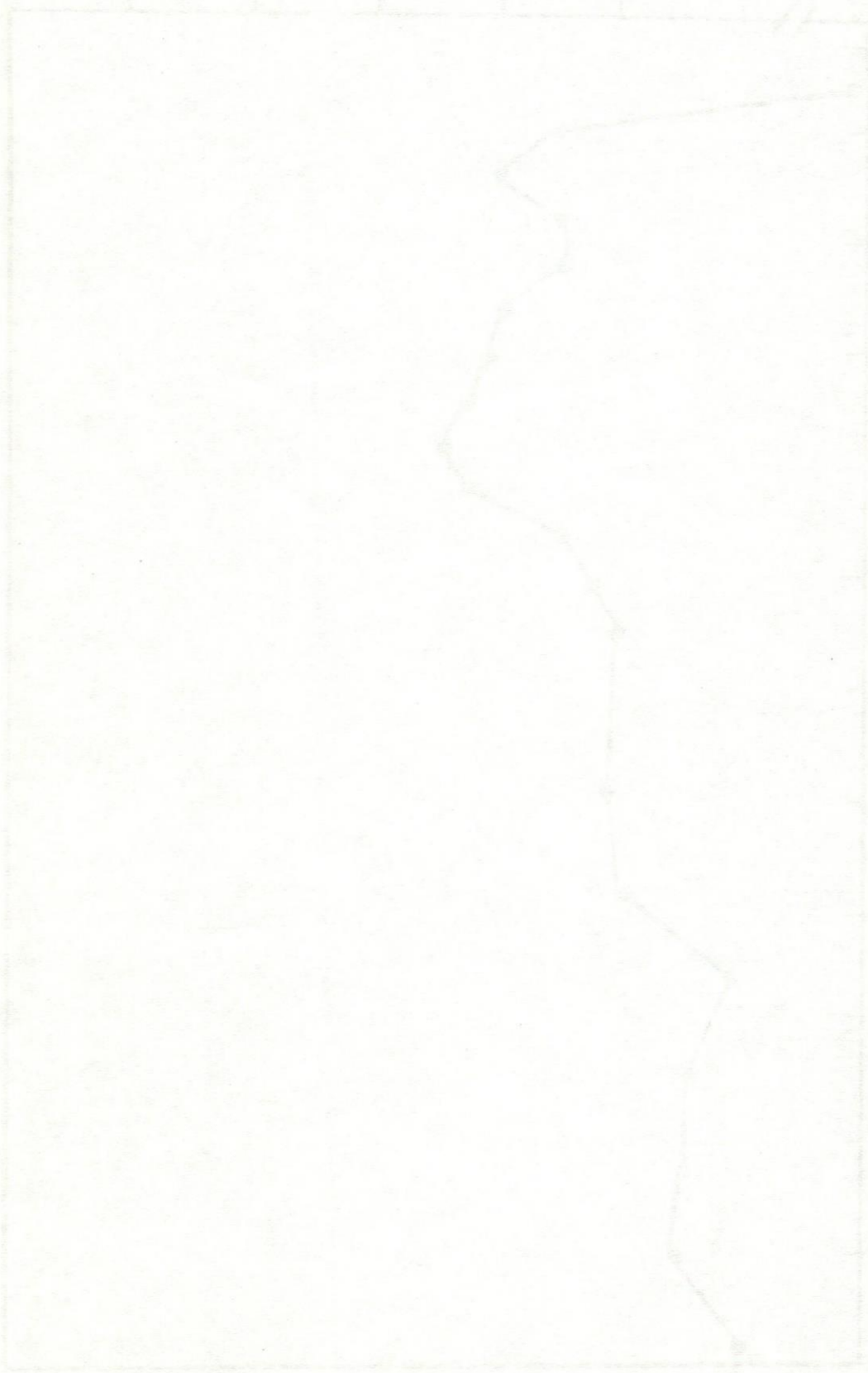


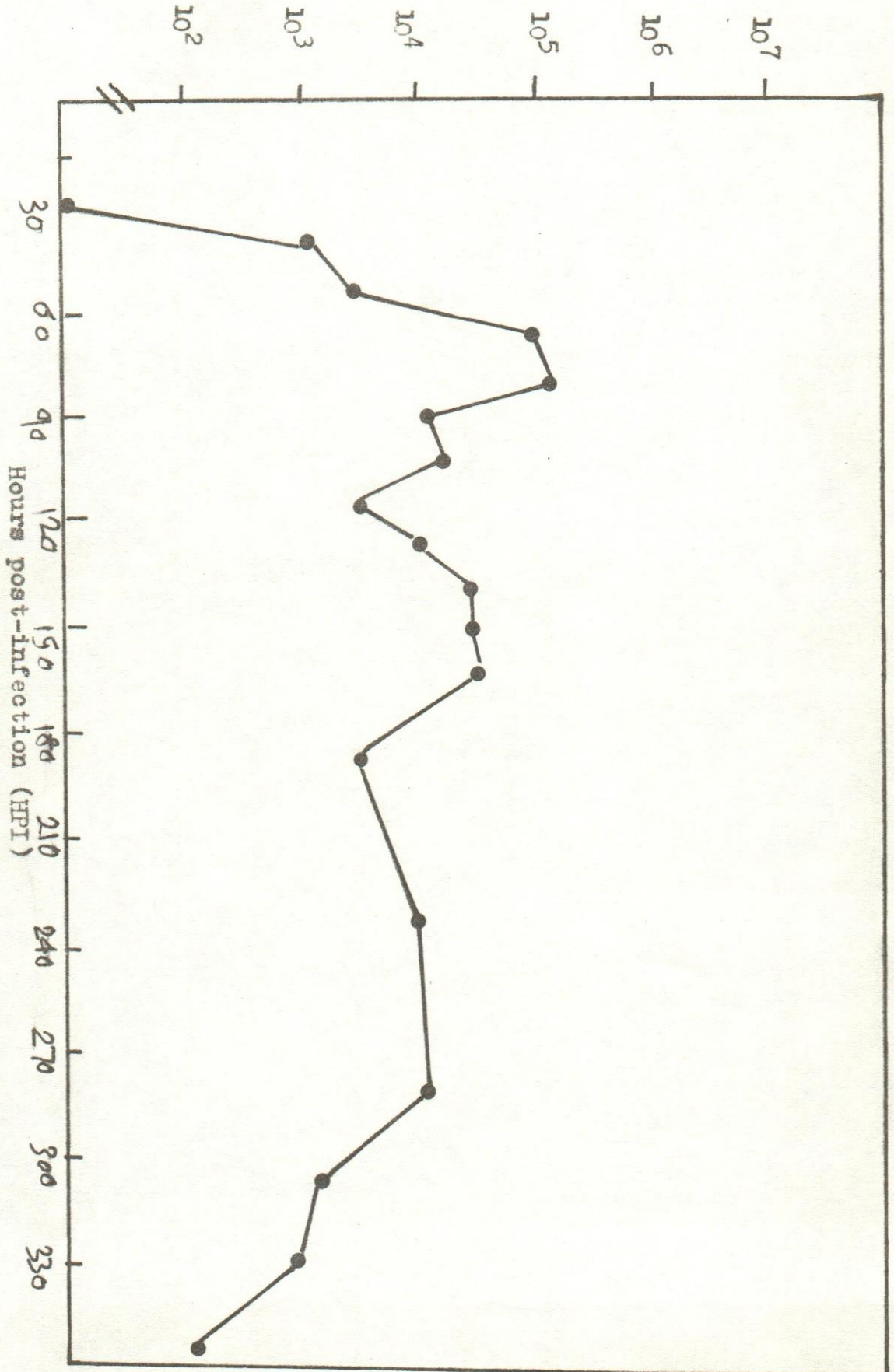
Figure (4): Titers of RVF virus in brains of mice collected at various intervals post-inoculation.



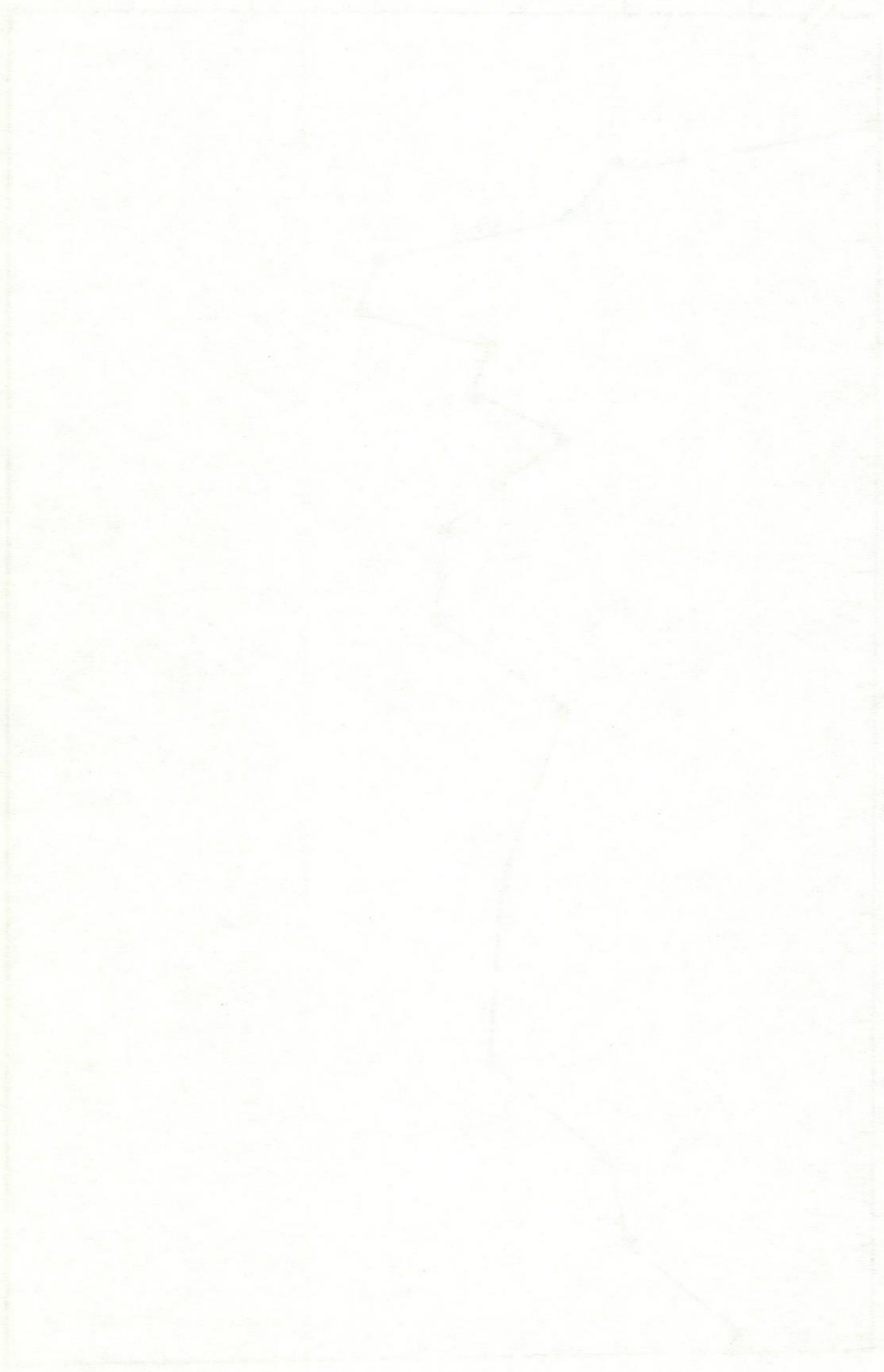
1957-58 Annual Report of the Board of Directors  
 The following information is for your information only.  
 The Board of Directors has approved the following  
 resolution:



Titer expressed in log TCID<sub>50</sub>/ml.



Figure(5): Titers of RVF virus in kidneys of mice collected at various intervals post-inoculation.



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