TITLE OF PROJECT

Biological Effects of Radiation, Metabolic and Replication Kinetics Alterations.

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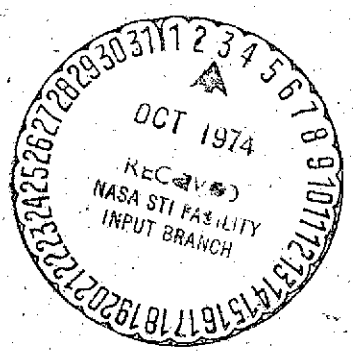


INTRODUCTION

During the life of this contract studies have been designed and carried out examining in vivo and in vitro the biological effects of radiation upon normal and cancerous tissues. Tritium (^3H), usually in the form of tritiated thymidine (^3HTdR), has been employed exclusively in these experiments. A macromolecular precursor of DNA, ^3HTdR is incorporated into the cell nucleus during synthesis and provides intranuclear beta radiation. Tritium-labeled cells were studied with autoradiographic methods; cell cycle kinetics were determined and cell function modified by radiation dosage or by drugs was also evaluated. The long term program has included: 1) the effects of radiation on cell replication and the correlation with incorporated dose levels, 2) radiation-induced changes in cell function, viz., the response of beta-irradiated spleen lymphocytes to antigenic stimulation by sheep red blood cells (SRBC), 3) the kinetics of tumor and normal cell replication and 4) megakaryocyte formation and modification by radiomimetic drugs. The results of some of these studies, having been published or described in previous annual reports, will only be summarized, and the others will be detailed.

I. Effects of Radiation on Cell Replication; Correlation with Incorporated Dose Levels.

Previous investigations have shown that increasing doses of ^3HTdR will disrupt the mitotic labeling (PLM) curves of rat hepatocytes, spleen lymphocytes or ileal crypt cells in vivo (1-4) and that the effects are dose-dependent. A similar experiment performed in vitro

with HeLaS$_3$ cells showed that increasing doses of ^{3}HTdR as a pulse label produced marked distortions of the PLM curve, which were dose-dependent. These were similar qualitatively to those changes induced in vivo. (Fig. 2-6). These findings eliminate the possibility of ^{3}HTdR re-utilization as a factor and establish the changes as effects of beta radiation.

Experiments to determine the amounts of radioactivity incorporated by the nuclei at the different dose levels were continued. Nuclei were isolated by density gradient methods; aliquots of equal volume were then taken for DNA determination, radioactivity assays in a liquid scintillation counter and particle counting in a Coulter counter. Incorporated activity was thus determined in rads per gram of nuclear DNA and then calculated for labeled nuclei from the labeling indices in autoradiographs. The results show that there is a good correlation between the labeling dose and the incorporated radioactivity (Table 1). The hepatocytes, after 1.0 μCi/gm absorbed an average radiation dose equivalent to 4.58×10^{-9} rads per day per labeled nucleus while the splenic lymphocytes showed an average absorption of 1.17×10^{-9} rads per day. The radioactivity in the HeLaS$_3$ cells rose from 1.44×10^{-10} rads after 0.01 μCi/lm to 1.49×10^{-7} rads after 10 μCi/ml.

As mentioned in previous reports the finding that the PLM curves of HeLaS$_3$ cells were altered by doses of ^{3}HTdR as small as 0.1 μCi/ml certainly suggests that in metabolic studies where radioactive precursors are employed the sensitivity of the biological specimens to radiation effects must be investigated.

II. Radiation Induced Changes in Cell Function.

The finding that the cycling of rat splenic lymphocytes was still pertubed 5 weeks after a dose of 10 μCI/gm, even after the disappearance of all labeling (4), raised the possibility of alteration of the genome of these lymphocytes. This in turn suggested that these cells might also have undergone changes in functional behaviour. The response of these cells to antigenic stimulation was tested.

As reported last year the labeling index of splenic lymphocytes from rats preteated with ^{3}HTdR, 10 μCi/gm, does not rise as briskly as that of controls following a challenging dose of sheep erythrocytes (SRBC). This has been interpreted as showing that the disturbance of cell kinetics by ^{3}HTdR dampens the synchronized increase in the number of cells entering DNA synthesis following SRBC.

A more specific index of functional change is the production of blood hemolysin after SRBC. This was studied in both preteated and control animals. Male Wistar rats 3 weeks old, were injected I.P. with ^{3}HTdR, 10 μCI/gm. Five weeks later they received 1 ml of 1% SRBC solution I.V. Blood samples were drawn from the respective animals at intervals of 1 to 10 days and analyzed quantitatively for levels of hemolysis. The same procedure was carried out in control 8 week-old animals who had not received any ^{3}HTdR. The data show that the control animals exhibit slowly rising titres that never reach above a mean of 1:52 (on day 5), and tend to plateau at very low levels through day 10 (Fig. 7) In the irradiated rats on the other hand, the titre rises on day 3, peaks sharply at 1:300 on day 4, diminishes gradually to a broad trough at 1:72 on days 8 and 9 and then peaks

again on day 10 to over 1:400 (Fig. 8). These results indicate that radiation has altered the response of the spleen lymphocytes. These studies have not been pursued because of lack of support. Thus, radiation-induced altered cell replication may be associated with other metabolic changes.

III. Normal and Tumor Cell Replication

A. Normal Cells.

The investigations of the effects of various drugs on cell cycling have been concluded. Studies were done with methotrexate, nitrogen mustard and hydrocortisone and the results were similar to those found with IUDR, namely alteration in the replication cycle with slowing of DNA synthesis, arrest in G_2 and reduction in the mitotic index. A reprint is enclosed.

The studies with germfree rats have also been concluded and showed that the mitotic labeling curves of spleen lymphocytes and ileal crypt cells are not altered by the sterile state. A reprint is enclosed.

B. Tumor Cells

Studies of the replication kinetics of tumor cells have continued and the movement of normal and tumor cells between proliferating and non-proliferating compartments has been examined using the methods described in our last report. The model has been the autogenous rat sarcoma produced with 3-methylcholanthrene (3-MC). The results show that only 40-60% of the initial cohort of cells in DNA synthesis (S) complete mitosis and enter G_1 to continue cycling.

About 20-35% of the S cohort leaves the cycle and stays in G_2 for long periods of time and 20-40% of the residual S cohort spends protracted times in G_1. The movement of cells is bidirectional, entering or leaving the cycle from either the proliferating or the non-proliferating compartments. The continual turnover of the compartments is a major determinant of tumor growth. Clinically this becomes a major factor in therapy. If a tumor has a large non-proliferating compartment the administration of cytotoxic agents designed to interfere with cell cycling will of necessity produce disappointing results.

Since the use of antitumor agents in man has been limited by their adverse effects on the coexistent normal replicating tissues such as the gastrointestinal tract or the hematopoietic system, dose levels have been maintained to avoid undue toxicity to the host tissue. It is conceivable therefore that many tumor as well as normal cells may escape the lethal damage of cytotoxins. Since the continued growth of the tumor is thus dependent on these surviving cells it was of interest to examine the proliferation pattern of tumor cells after one of these drugs.

Rats with 3-MC-induced sarcomas received nitrogen mustard (HN_2), 0.1 mgm/kg, daily for varying periods up to 7 days. Four groups of experiments were performed and cell proliferation was studied with continuous infusions of ^{3}HTdR (See reprint). Tumor growth stopped during HN_2 administration with a fall in mitotic indices, and mitotic and interphase labeling rates. Most mitoses were unlabeled, derived from G_{2_0} cells. Tumor growth resumed after withdrawal of HN_2, the mitotic index and labeling rate returning to pretreatment levels. Interphase labeling lagged behind however, and thus it appeared that the pre-

treatment growth rate was associated with a reduced proliferating pool.

Of further interest to us has been the general observation that most antitumor agents have been tested for their cell-killing abilities or cycle-disrupting affinities in in vitro systems. In order to achieve a therapeutic effect of reasonable value in the host it is implicit in such studies that tumor cell replication in vivo be similar to that found in tissue culture. Accordingly tumor cells were taken from samples of four individual 3-MC induced autogenous rat sarcomas and grown in tissue culture. They were pulse-labeled with ^{3}HTdR, 0.1 μCi/ml, PIM curves were derived and the replication kinetics of the tumor cells grown in vitro were determined. The cycle time (Tc) was about 17 hours, S was about 6 hours and G_1 was estimated at 6-8 hours (Fig. 9). In the tumor grown in vivo, Tc was 40 hours, S was 24 hours and G_1 was about 14 hours (5). The data indicate the obvious differences between cell replication in vitro and in vivo and emphasize the importance of establishing a more effective method for choosing and testing drugs with potential tumoricidal properties.

We wish to thank the National Aeronautics and Space Agency for the continued support that enabled us over the past several years to continue investigations in the field of radiation research.

PUBLICATIONS

1. Sklarew, R. J., Pachter, B., Hoffman, J., and Post J. Formation of Splenic Megakaryocytes in Young Rat Exp. Cell Res. 1971 64, 195-203.

2. Post, J., and Hoffman, J. - Growth Kinetics of Solid Tumors in Pathobiology Annual 1972, Editor: H. L. Iochim, Appleton - Century - Crofts, New York City - 1972.

3. Hoffman, J., and Post, J. - Effects of Nitrogen Mustard, Methotrexate and Hydrocortisone on Cell Replication in Rat Ileum and Spleen.
Proc. Soc. Exp. Biol and Med. 1972 140, 444-448.

4. Post, J., Wilson, R., Sklarew, R. J., Pachter, B., and Hoffman, J. Replication of Spleen Lymphocytes and Ileal Crypt Cells in Conventional and Germfree Young Rats
Proc. Soc. Exp. Biol. and Med. 1972 140, 553-555

5. Post, J., Sklarew, R. J., and Hoffman, J. - Cell Proliferation Patterns in An Autogenous Rat Sarcoma.
Jour. Nat. Cancer Inst. 1973 50, 403-414.

6. Hoffman, J. and Post, J. - Effects of Antitumor Drugs on Cell Cycle in Drugs and the Cell Cycle.
Editor: Zimmerman, A. M., Padilla, G. M., and Cameron, I.
Academic Press, New York City, 1973.

7. Post, J., Sklarew, R. J., and Hoffman, J. - Immediate and Delayed Effects of Nitrogen Mustard on Cell Replication in An Autogenous Rat Sarcoma
Jour. Nat. Cancer Inst. 1974, 52, 1897-1903.

REFERENCES

1. J. Post and J. Hoffman Rad. Res. 1965 26, 422
2. J. Post and J. Hoffman Rad. Res. 1967 30, 748
3. J. Post and J. Hoffman Rad. Res. 1968 34, 570
4. J. Post and J. Hoffman Rad. Res. 1971 45, 335
5. J. Post and J. Hoffman Brit. J. Cancer 1968 22, 149

FIGURE LEGENDS

Fig. 1. Mitotic Labeling Curves - hepatocytes and spleen lymphocytes.

Fig. 2. Mitotic Labeling Curve - $HeLaS_3$ - 0.01 μCi/ml.

Fig. 3. Mitotic Labeling Curve - $HeLaS_3$ - 0.1 μCi/ml.

Fig. 4. Mitotic Labeling Curve - $HeLaS_3$ - 1.0 μCi/ml.

Fig. 5. Mitotic Labeling Curve - $HeLaS_3$ - 2.0 μCi/ml.

Fig. 6. Mitotic Labeling Curve - $HeLaS_3$ - 10. μCi/ml.

Fig. 7. Hemolysin Response to SRBC - Non-radiated rats

Fig. 8. Hemolysin Response to SRBC - rats treated with ^{3}HTdR, 10 μCi/gm.

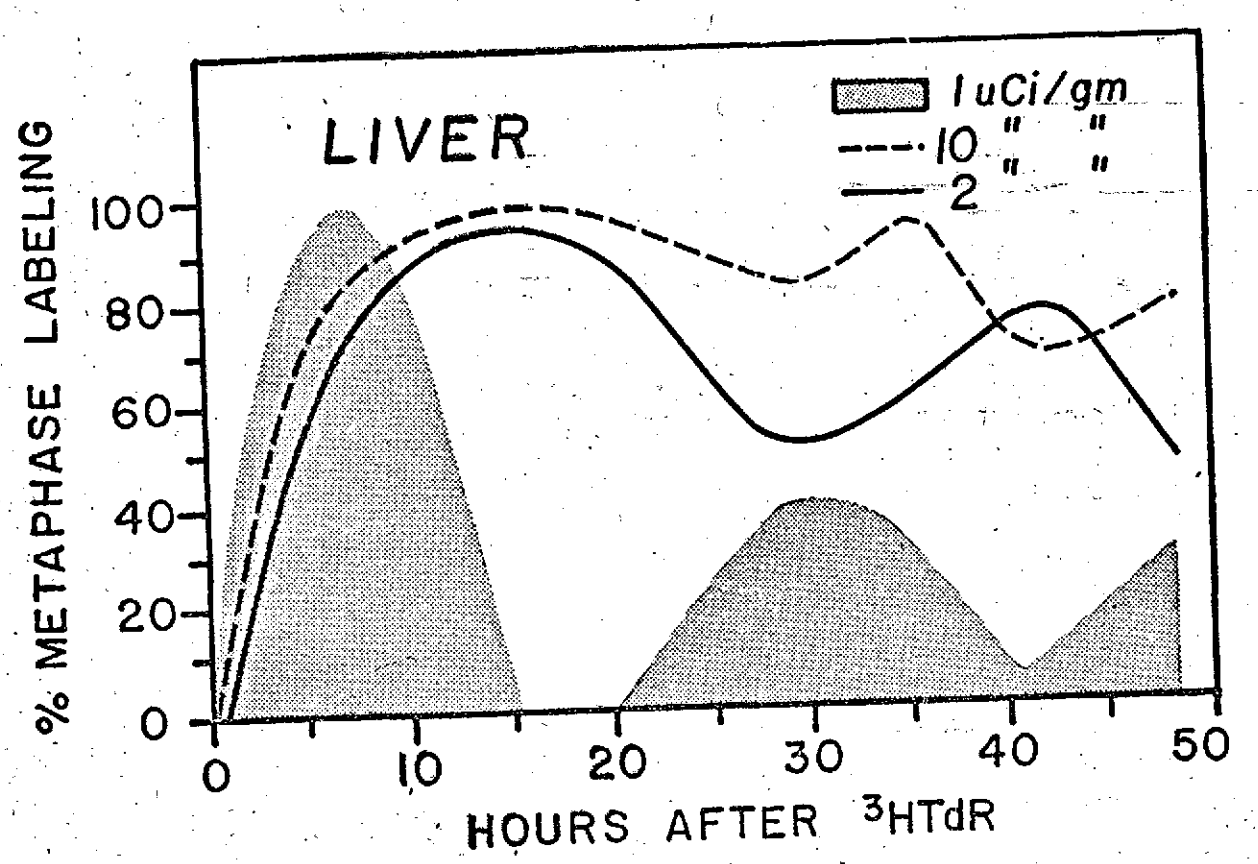
Fig. 9. Mitotic Labeling Curve - MCA - Induced Rat Sarcoma Cells grown in vitro.

TABLE 1

^{3}HTdR Labeling Dose and Incorporated Radioactivity

Tissue	Dose	Pulse	DPM Per Nucleus X 10^{-3}	Labeling Index (%)	DPM Per Labeled Nucleus	Rads/day Per gm Labeled Nuclear DNA		Rads/day Per Labeled Nucleus
Liver	1.0 µCi/gm	4 hrs.	1.09	3.0	.036	374.4		4.58×10^{-9}
	2.0 µci/gm	4 hrs.	2.40	3.1	.077	806.4		9.87×10^{-9}
	10.0 µCi/gm	4 hrs.	15.50	6.7	.23	2,419.2		2.96×10^{-8}
Spleen	1.0 µCi/gm	4 hrs.	4.2	46.7	.009	70.6		1.17×10^{-9}
	2.0 µCi/gm	4 hrs.	6.14	46.8	.013	100.8		1.67×10^{-9}
	1.0 µCi/gm	4 hrs.	42.8	44.5	.096	745.9		1.24×10^{-8}
HeLaS$_3$	.01 µCi/ml	20 min.	3.36×10^{-4}	30	1.12×10^{-3}	6.29		1.44×10^{-10}
	.10 µCi/ml	20 min.	3.14×10^{-3}	30	1.07×10^{-2}	60.04		1.38×10^{-9}
	1.0 µCi/ml	20 min.	2.58×10^{-2}	30	8.6×10^{-2}	482.4		1.11×10^{-8}
	2.0 µCi/ml	20 min.	6.41×10^{-2}	30	2.14×10^{-1}	1202.4		2.76×10^{-8}
	10.0 µCi/ml	20 min.	3.44×10^{-1}	30	1.15	6465.6		1.49×10^{-7}

Fig. 1



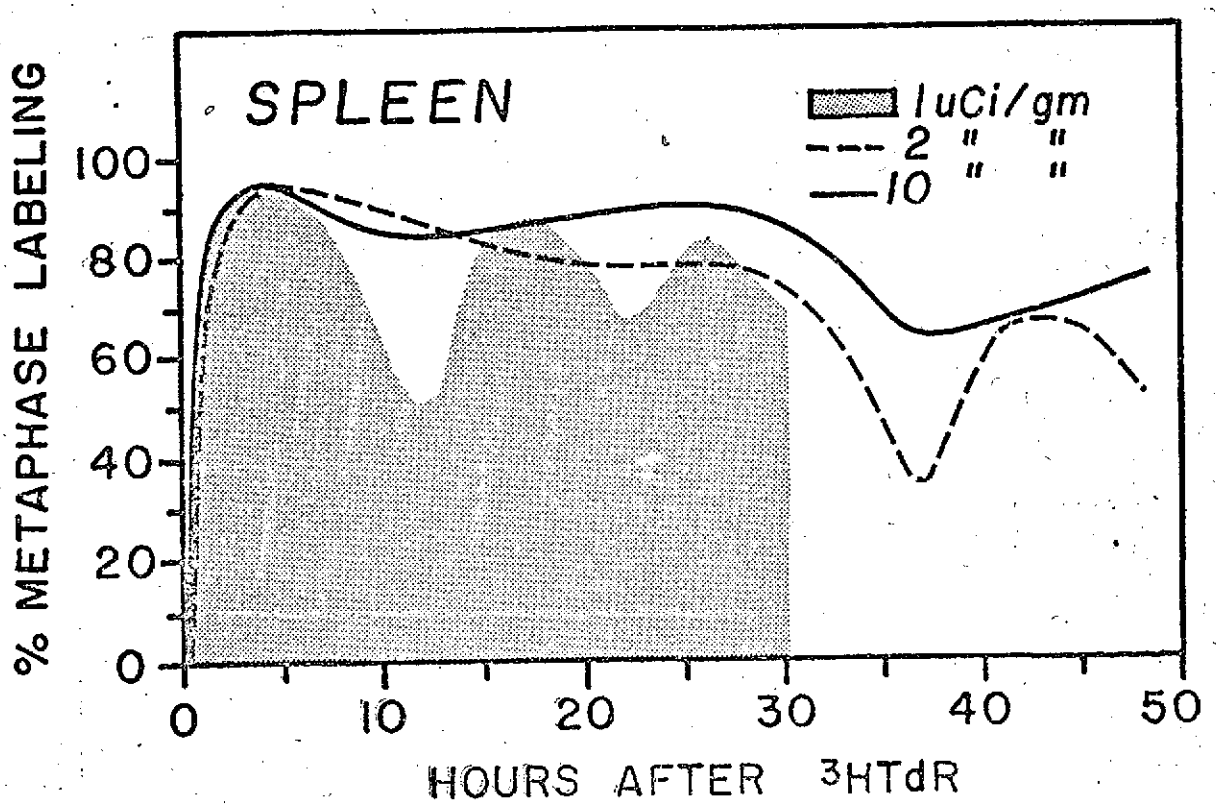


Fig. 2

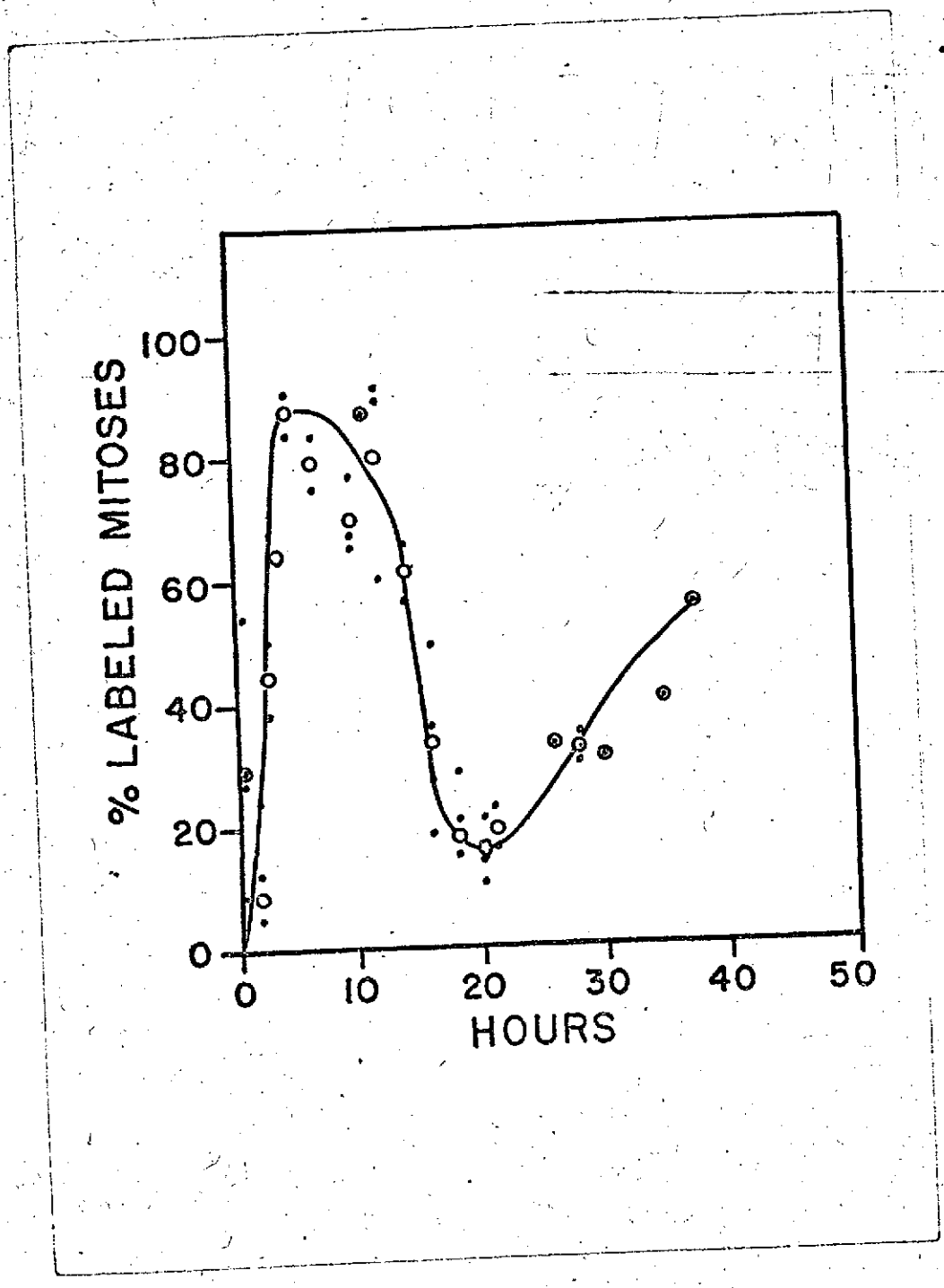


Fig. 3

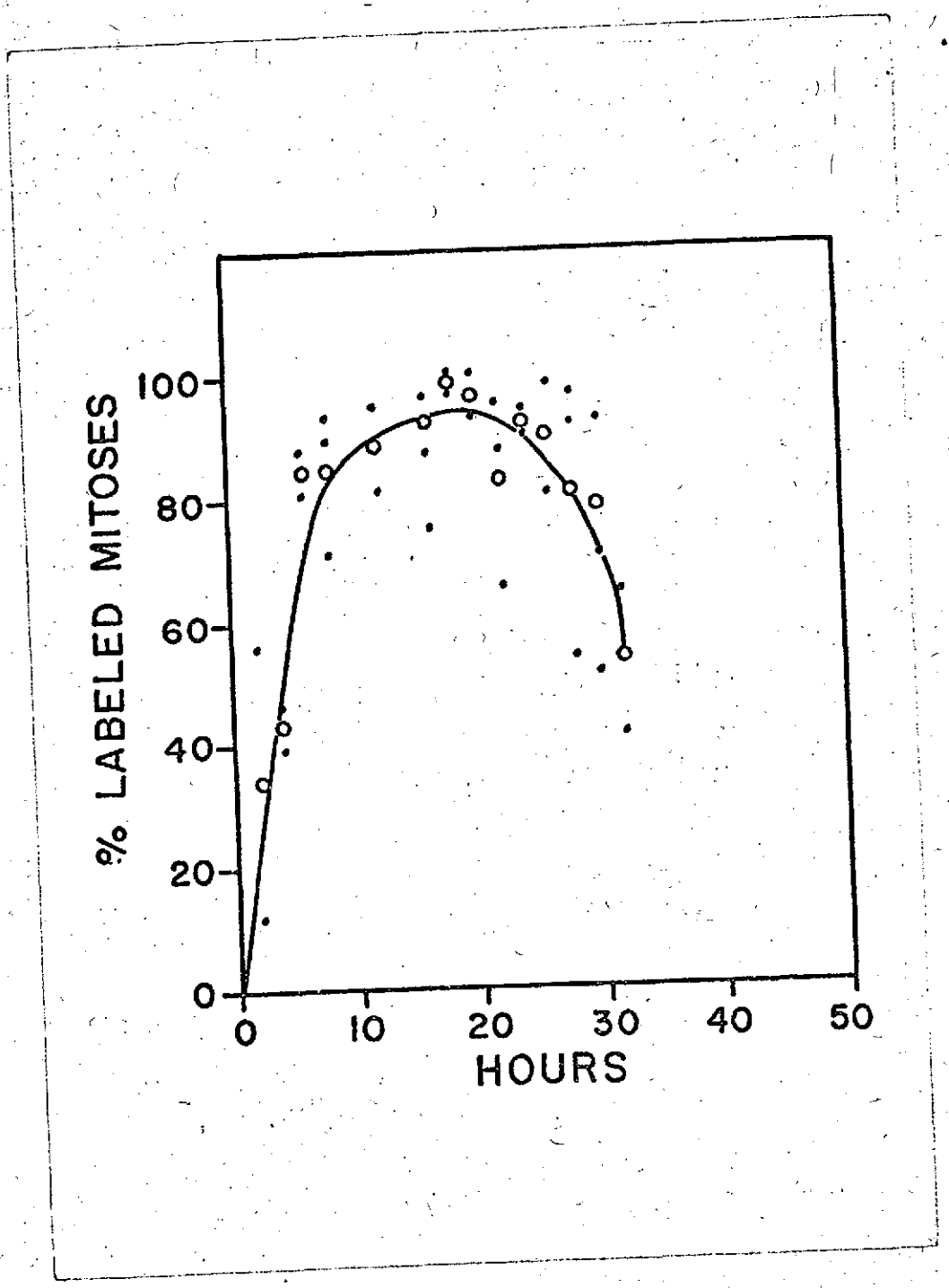


Fig. 4

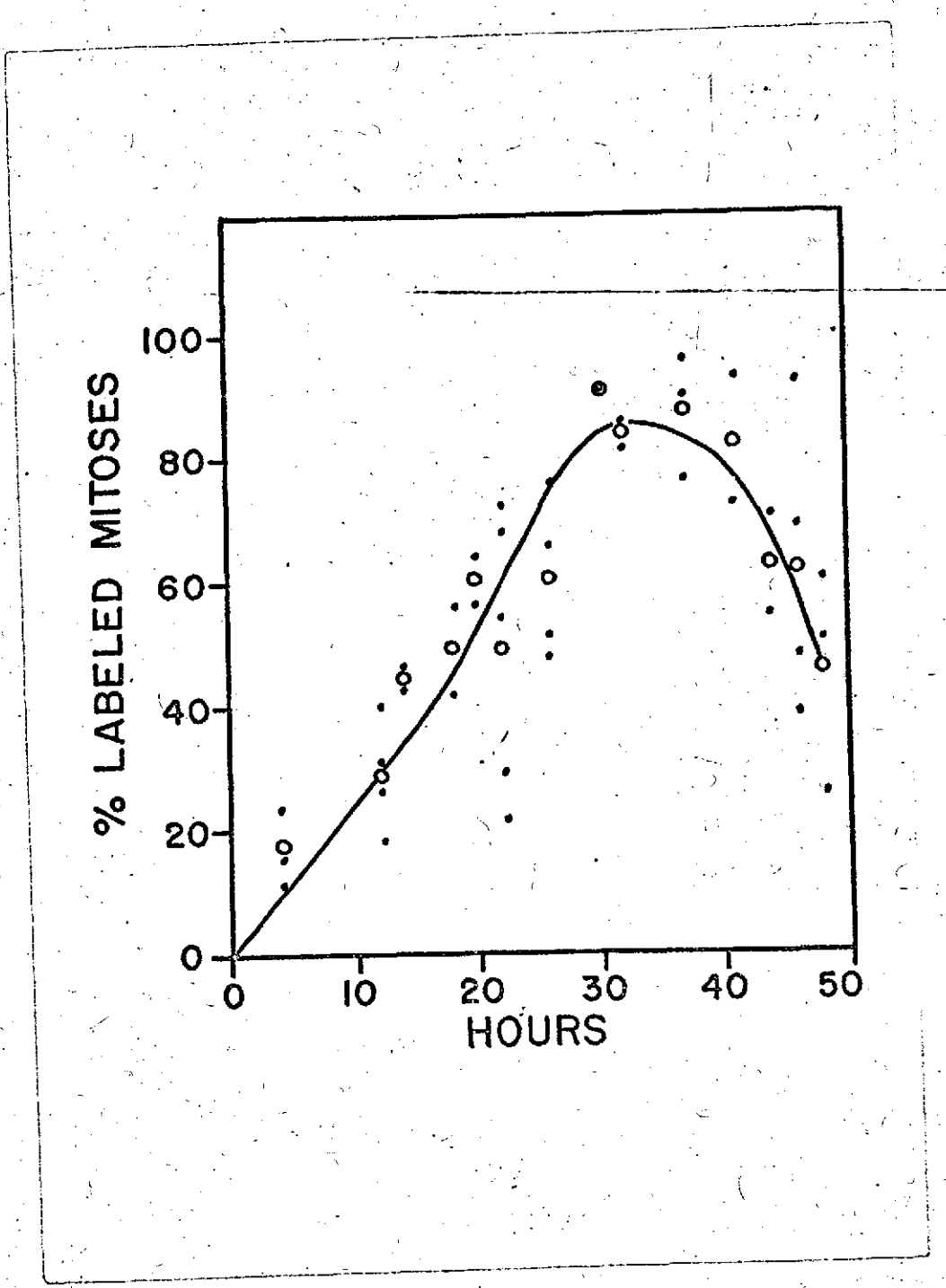


Fig. 5

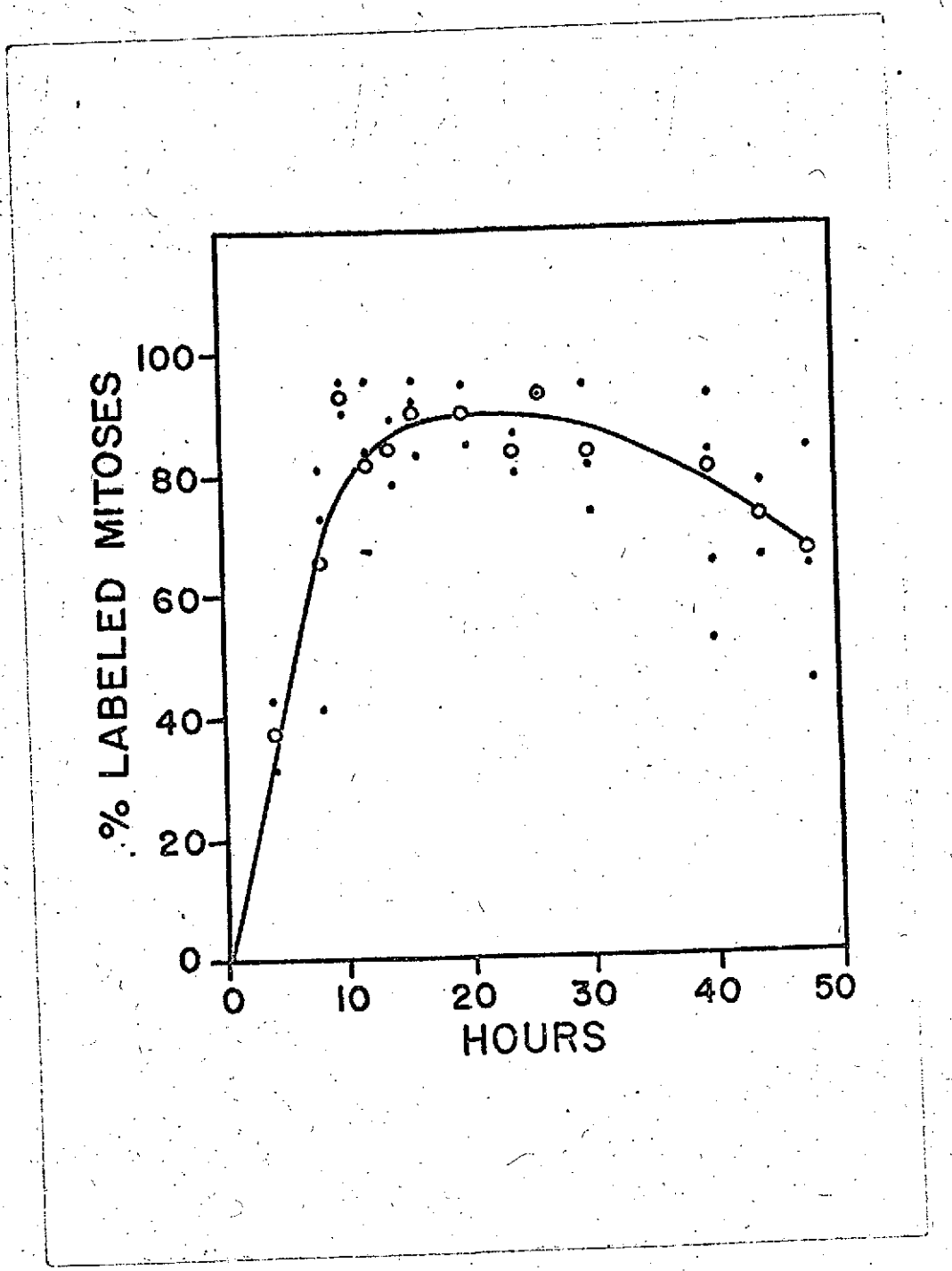


Fig. 6

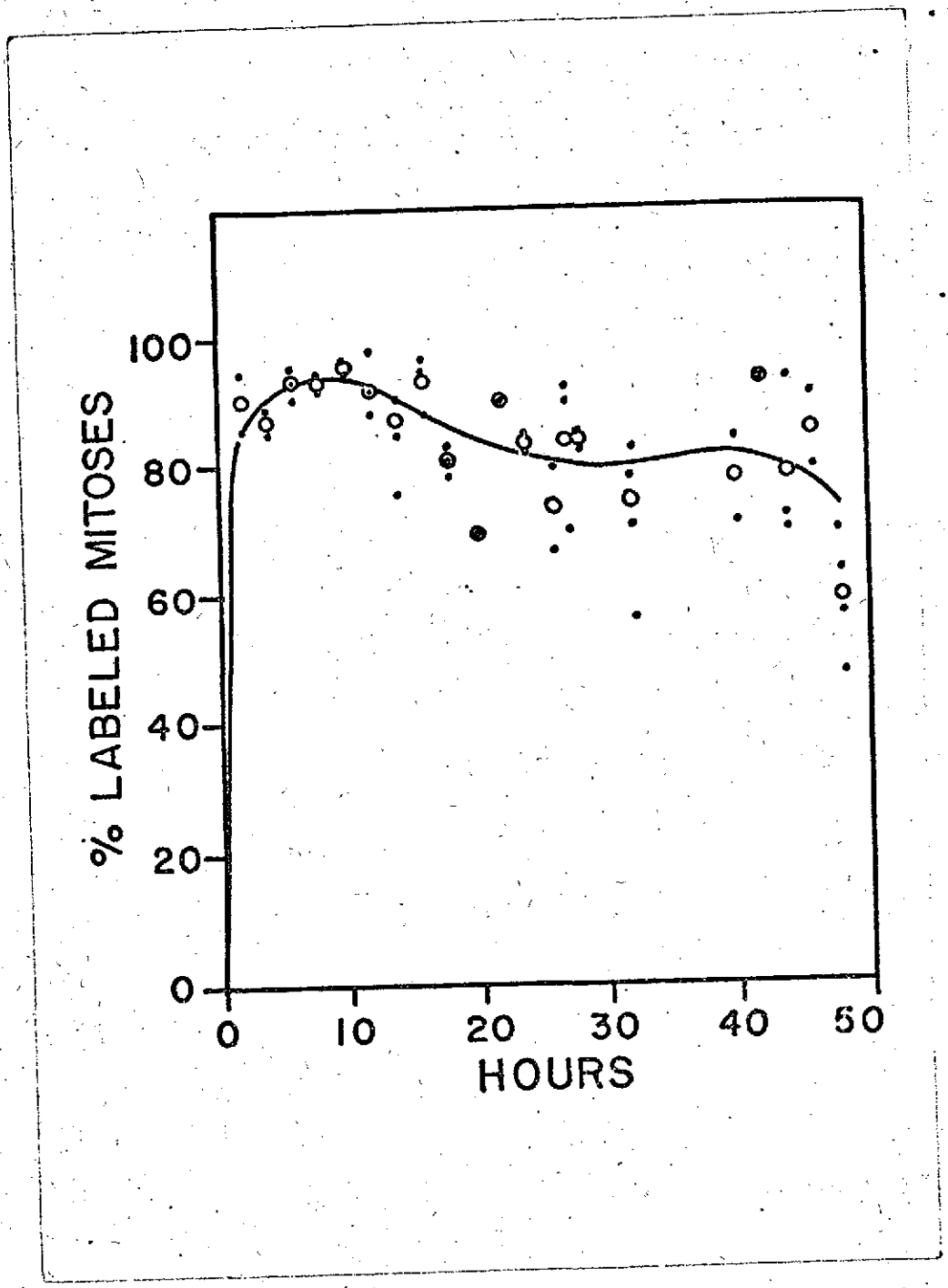


FIG. 7: CONTROL RATS - NO RADIATION

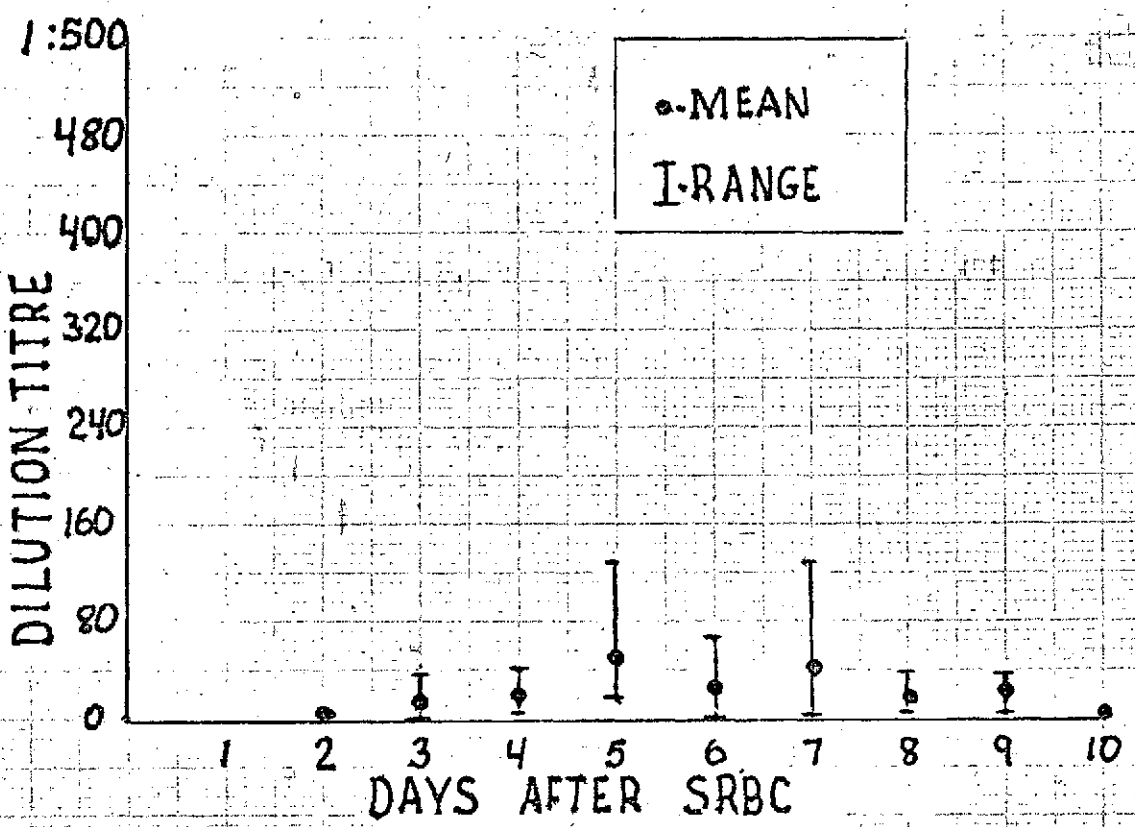


FIG. 8: RATS TREATED WITH ^{3}HTdR (10 μCi/gm.)

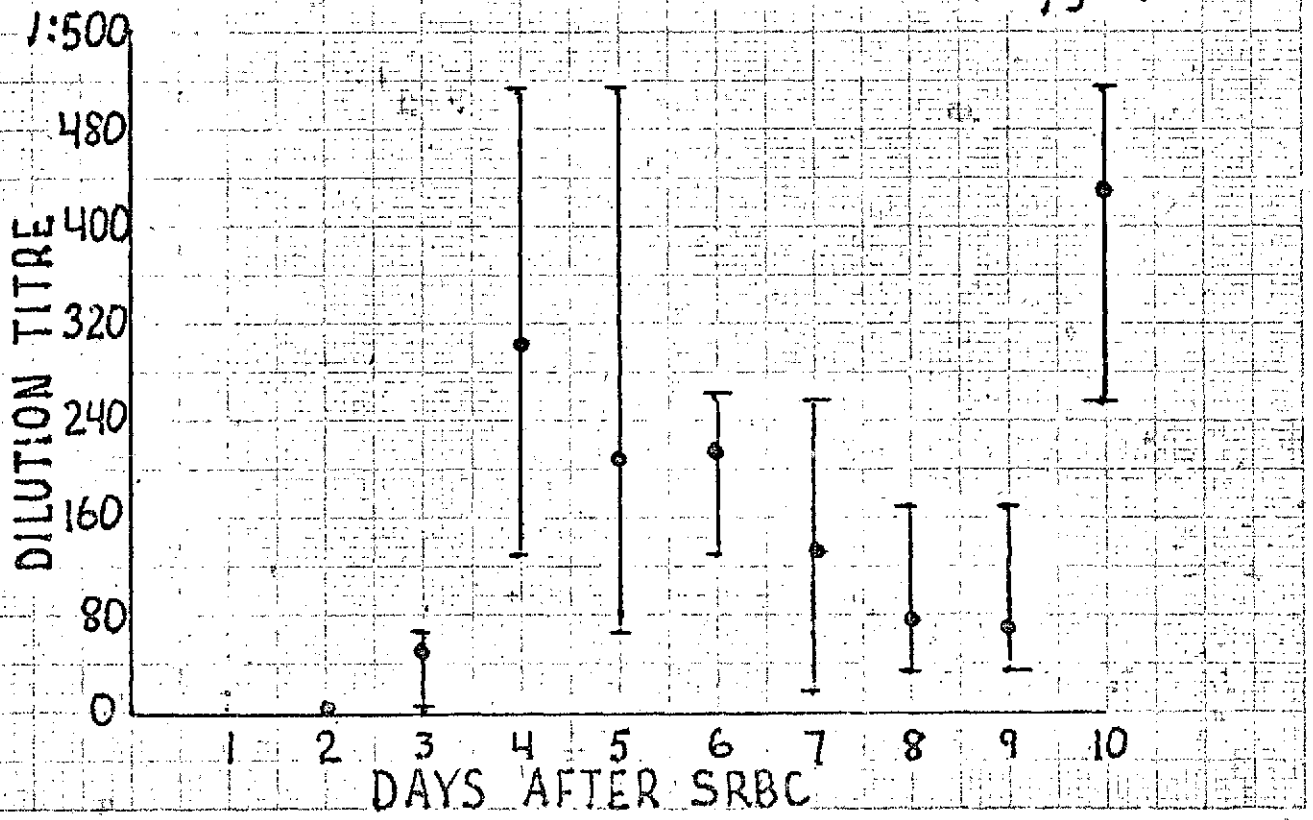


Fig. 9