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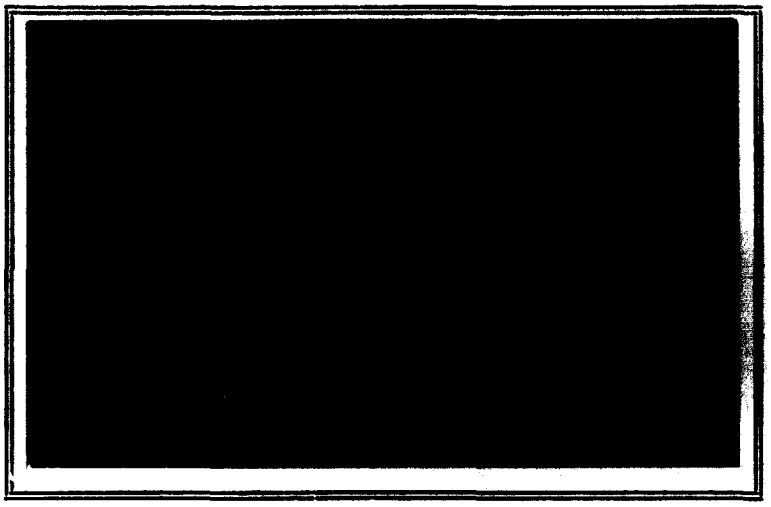

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Effects of sublethal doses of gamma radiation on the developing rat brain

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Abstract

Newborn rats were irradiated with ^{60}Co gamma rays. Doses of 0, 80 or 160 rads were given to the whole body. The whole body and brain weights, DNA and RNA contents of the brain and ^{3}H-thymidine or ^{3}H-uridine incorporated by the brain were measured at 5, 10 or 15 days after birth. A dose of 160 rads produced clear alterations in the brain but no clear effects could be detected when 80 rads were given.

Introduction

Doses of 10-40 rads of X-rays given to newborn rats produce abnormalities in individual neurons of the brain and affect their organization (11). 100-200 rads are usually needed to cause clearly demonstrable cell death in cerebrum (7) or cerebellum (1). Reported studies of DNA and RNA in the developing brain after doses lower than 200 rads are few. 100 rads given to two-day-old rats can produce a depression of ^{3}H-5-uridine incorporated in the brain at seven days of age (6). No attempts to correlate known meorphological distrubances prodeced in the brain by doses lower than 100 rads to changes in nucleic acid synthesis are known to the authors. The aim of this work is to give some quantitative information in this aspect that could serve as a basis for the planning of more detailed studies necessary to permit causal analysis. The brain of rats irradiated at birth was studied within 15 days. Particular attention was paid to DNA and RNA kinetics.

Materials and Methods

Animals: Wiatar rats were from the department animal breeding colony. At birth each litter was reduced to eight or six animals. No distinction was made between males and females. Body weight was measured before injection. Brain tissues were weighed before homogenization.

Irradiation procedures: Gamma radiation was administered with a freely radiating ^{60}Co source (12) at a dose rate of 10 rad/min as measured by ferrous sulphate dosimetry and TLD dosimetry. The rats were placed in plastic tubes with 2 mm thick walls and kept rotating around the gamma source as well as around their own axis. All irradiations were performed between 12 and 24 hours after birth.

Radioactive Compounds: Methyl-^{3}H-thymidine (^{3}H-TdR) and 5-^{3}H-uridine (^{3}H-UR) from Amersham, England, were used. Specific acitivity was 5 Ci/mmol and concentrations were obtained by dilution in both compounds. The required concentrations were obtained by dilution in 0.9% NaCl.

Separation of tissues: The animals were killed by decapitation. The brains were divided in two parts, telencephalon + diencephalon (the "TD part") and metencephalon + mesencephalon + medulla oblongata (the "MMM part"). These structures were immediately removed and frozen on solid CO_2 and thereafter stored at -20^{o}C until analysed.

Homogenization was performed for 30 x 3 (times x sec) at 1500 rpm by a motor driven Potter-Elvehjelm glass-teflon homogenizator.

DNA and RNA measurements: DNA and RNA were extracted by a modified Schmidt and Thannhauser method as outlined in Table 1. The amount of DNA was estimated by the diphenylamine method modified by Burton (4) with calf thymus DNA (SIGMA) as a standard. The RNA content was estimated by the orcinol reaction (9) with yeast RNA (SIGMA) as a standard. Absorbancy was measured in a double beam spectrophotometer (Shimadzu) at 600 nm (DNA) and 670 nm (RNA).

Activity Measurements: ^{3}H activity was determined in a Nuclear Chicago Mark II scintillator counter. One ml of sample was mixed with 10 ml of the scintillator cocktail containing 7 g PPO and 0.35 g POPOP in 0.7 l toluene and 0.3 l Triton X-100. For samples prepared in NCS solubilizer (see below), a cocktail containing 6 g/l PPO and 75 mg/l POPOP in toluene was employed.

Incorporation of ^{3}H-TdR and ^{3}H-UR versus time: Experiments were first performed to investigate at which time after injection maximal incorporation in DNA and RNA of subcutaneously injected ^{3}H-TdR and ^{3}H-UR is obtained in the brain. In the cases of ^{3}H-TdR, forty animals (6 or 16 days old) were divided in 10 groups (4 animals in each) and injected with 0.5 or 1.0 µCi per gram body weight. Similarly, 2.0 µCi ^{3}H-UR per gram body weight was injected subcutaneously in 36 animals (3 or 14 days old) divided in 9 groups (4 animals in each). The animals were killed by decapitation at different times after injection.

The brains were removed and homogenized and the ^{3}H-activity was measured in the non-acid soluble fraction (see Table I), which were pretreated with NCS solubilizer for 15 hours at 40°C.

Irradiation schedule: Irradiation was made on whole litters. The doses were 0, 80 and 160 rads and given within 12 to 24 hours after birth. The animals were killed at 5, 10 or 15 days after birth. The schedule chosen has the disadvantage that differences in, for example, the mean DNA and RNA content of the unirradiated brain between litters are not possible to correct for. On the other hand, the risk of discrimination by the mother between irradiated and non-irradiated animals is eliminated.

Results

Preliminary experiments

The incorporation of ^{3}H-TdR and ^{3}H-UR by the brain was studied in order to establish the amounts to be injected in the main experiment and an appropriate time interval between injection and killing of the animals.

Incorporation of ^{3}H-TdR. The incorporated ^{3}H-activity reached a plateau 2 hours after injection of 0.5 µCi ^{3}H-TdR/g (Fig. 1 a). Similar results were found in 6 and 16 days old animals. However, when 1.0 µCi ^{3}H-TdR/g had been injected, ^{3}H-TdR was still being incorporated after four hours.

Incorporation of ^{3}H-UR. The incorporation rate of ^{3}H-UR decreased very rapidly after 2 hours and both in 3 and 14 days old animals a plateau began about 4 hours after injection (Fig. 1 b), the amount of precursor injected being proportional to the amount incorporated (Fig. 2).

^{3}H-TdR and ^{3}H-UR metabolism at different ages of the rat. A study of the clearance of ^{3}H-TdR and ^{3}H-UR from the blood has been done for rats of 5, 15 and 30 days of age. That work is reported elsewhere (5) but the most important results are given here.

Clearance in the blood of subcutaneously injected ^{3}H-TdR and ^{3}H-UR follows neraly exponential functions. The precursor availability (PA) was defined as the integral of these exponential curves. The PA values can be interpreted as correction factors to be used when incorporation of precursors is compared for different developmental stages of rat tissues. This correction is probably adequate for most tissues when DNA and RNA synthesis are studied. However, for the brain it is also important to consider the possible existence of a changing blood-brain barriersystem, which not has been investigated by us.

Main experiments

Radiation effects on body and brain weight. No effect on body weight was

observed during the first 30 days after birth for rats irradiated with 160 rad 12-24 hours after birth (Fig. 3). However, the brain weight was found to be less than control values in all the animals irradiated with 160 rad (Fig 4). The relative weight deficit increased with age to about 13 % for both TD (telecephalon + diencephalon) and MMM (metencephalon + + mesencephalon + medulla oblongata) parts of the brain. For 80 rad, however, the weight decrease was slight.

Radiation effects on DNA and RNA content. The DNA and RNA contents in the normal developing rat brain are given in Fig. 5. A general agreement is found between our results and the results reported by other authors.

The DNA and RNA contents for irradiated animals at 5, 10 and 15 days of age can be seen in Fig. 6. Bothe the TD and the MMM parts from the irradiated animals show DNA values under the control values in 5 and 15 days old animals.

In the MMM part the relative DNA decrease was largest 5 days after birth (about 30 % with 160 rad) while in the TD part the DNA deficit was largest in 15 days old animals (about 15 % with 160 rad). The RNA curves (Figs. 6c, and 6d) shows little or no effect of the radiation in 5 days old animals. In older animals the MMM part showed a RNA decrease which was similar to the DNA decrease.

Radiation effects on incorporation of thymidine and uridine. The activity measured in the DNA and RNA fractions, which was normalized against brain weight or against DNA or RNA content is shown in Figs. 7 and 8. No clear radiation effects can be seen. However, all ^{3}H-TdR values for irradiated animals were somewhat lower than the control values at 15 days after birth. Each point in these curves stems from measurement on one litter

while the measurements in the preceeding section (DNA, RNA and weight) were done on two litters.

Discussion

Brain and body weight. It seems that doses of 160 rads given to the whole body of newborn rats do not significantly alter body weight during the first postnatal month. Brain weight, however, appears to be clearly affected by 160 rad while 80 rad produces a slight effect. It is interesting that the absolute weight deficit increased with age in the analysed parts of the brain when the dose of 160 rad had been applied. This is a parallel to the increasing weight deficit that occurs in rats that have been underfed a short period after birth (10). This tendency could not be observed after irradiation with 80 rad. Altman et coll. (2) found a weight decrease in cerebellum of 10-days-old rats given 200 rad by local irradiation at birth. They also observed neonatally irradiated animals 30 and 90 days after birth and found that both in weight and, with the morphological methods employed, the cerebellum seemed to have recovered completely, after 200 or 400 rads (3). Korogodina et al. (13) also found that histological recovery took place in cerebella irradiated with 200 rad five days after birth. They found, however, a weight deficit in cerebellum in 37 day-old animals. Valcana et coll. (17) found that even when 500 rad had been given to two-day-old rats a recovery pattern was observed although it was not complete.

Radiation effects on DNA and RNA content. From DNA measurements it may be seen if a radiation effect on brain weight reflects a decrease in cell number or not. A comparison between Figs. 4 and 6 shows that weight and DNA effects follow the same pattern in the MMM part, while this is not clear for the TD part. The effect on DNA is relatively small for the TD part so it is likely that the average mass per cell or the extracellular mass (e.g. water) has decreased. For the MMM part, however, the DNA decrease indicates that radiation interferes with cellproduction.

These findings are not surprising because most of the cell proliferation

in the TD part occurs before birth while cell differentiation goes on in this part for several weeks after birth. Whole body irradiation given at birth can therefore interact with the hypertrophic effect during differentiaition. The MMM part (especially cerebellum) on the other side has its bulk proliferation after birth which means that radiation interaction with cell production is likely to occur in our case.

The relative RNA decrease in the MMM part was of the same order as the DNA decrease at both dose levels for 10 and 15 days old animals. This indicates that the RNA content per cell does not change within the age interval studied.

It must be emphasized that these over-all measurements may hide important effects in cells which are few in number but that physiologically can be very important. Important changes in the amount of mRNA may also be hidden in the bulk RNA measurements.

Radiation effects on incorporation of radioactive thymidine and uridine.

Since no effects were reported in the availability of ^{3}H-TdR and ^{3}H-UR after irradiation of young rats (5), we have not performed any corrections due to the varying PA given in the preceeding section.

No clear radiation effects on thymidine and uridine incorporation could be seen on the 5, 10 or 15 days old rats irradiated at birth. It can thus be stated that it probably is difficult to see effects on precursor incorporation in the narrow "four-hour-window" many days after irradiation. For long term effects it is possibly more fuitful to look upon weight and total DNA and RNA content parameters that "integrate" disturbances over longer periods.

The liver and most other organs develop during the age intervals in which the experiments were performed. This means that differences in breakdown

of the injected precursors can occur. It is indeed true that the breakdown of ^{3}H-TdR and ^{3}H-UR changes during the studied ages but it has been shown that the amount of breakdown is not appreciable altered after irradiation with 160 rad (5).

A blood brain barrier system for nucleosides may develop and continously change during the studied growth period. Radiation-induced alterations in such a system also render conclusions about measured changes in the rate of DNA and RNA synthesis difficult. It can thus be stated that measurements of disturbances in precursor incorporation involve unknown factors that must be carefully investigated.

Conclusions

Our results do not permit any conclusions to be made about the mechanisms involved in radiotion-induced retardation of developing brain tissues. The slight changes in brain weight and DNA content demonstrated may even be due to general metabolic disturbances in the whole-body irradiated animal related to the gastrointestinal tract. However, the results do not exclude pronounced effects on DNA or RNA kinetics to occur in particular cell populations of the brain. A differential analysis of the effects of low dose irradiation must therefore be performed aiming at understanding of effects on different cell populations or different macromolecular fractions.

Acknowledgements

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Table 1. Outline of the modified Schmidt and Tannhauser (16) method used in this work.

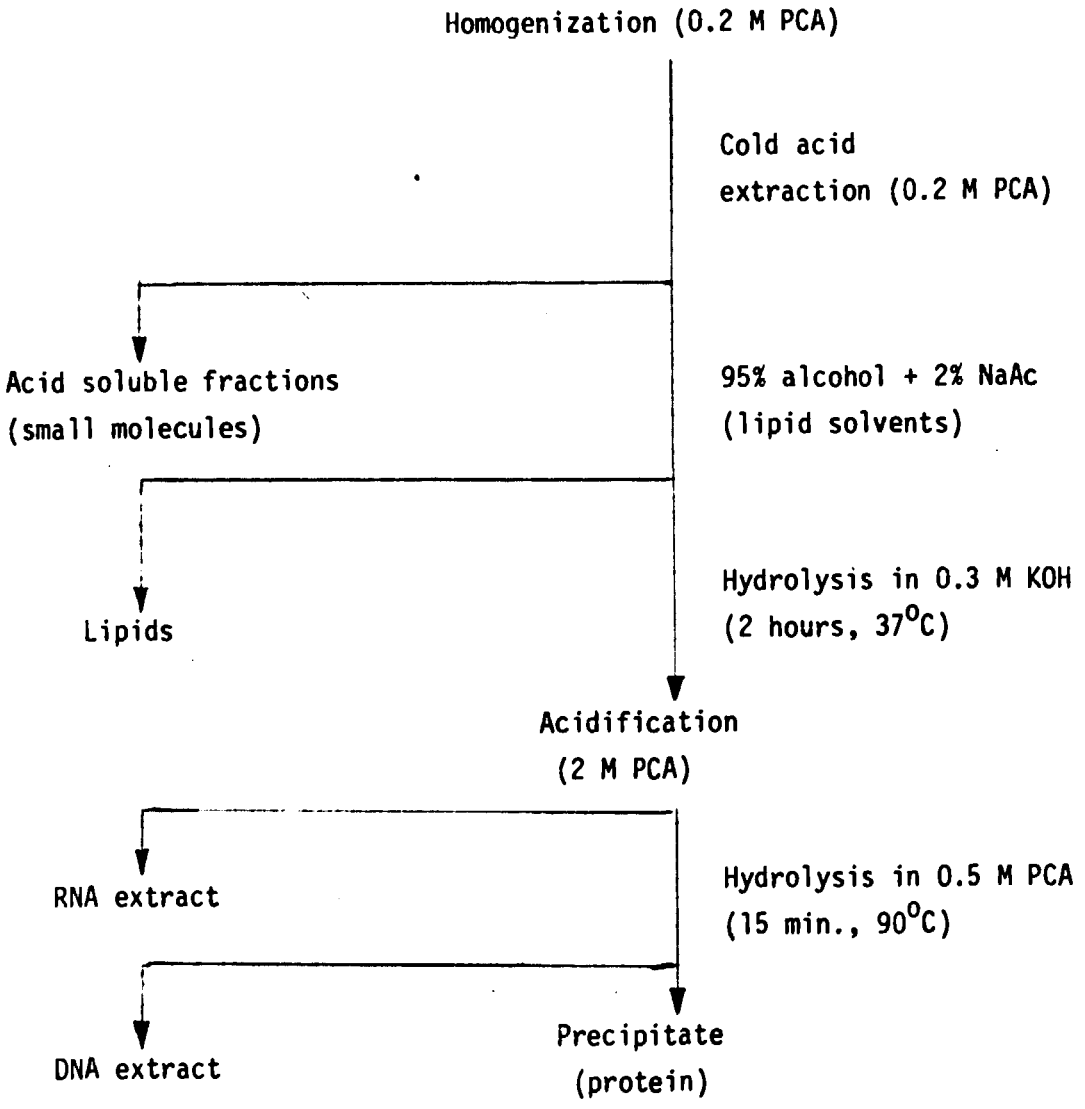


Figure texts

Fig. 1 Incorporation of subcutaneously injected ^{3}H-thymidine (a) and ^{3}H-uridine (b) as a function of time. Analysis was done on whole brains. In Fig. 1a the age of the animals were 6 (o) and 16 (o) days while in Fig. 1b the animals were 3 (o) and 14 (o) days old. Each point represents measurements on 3-6 animals. Different amounts of radioacrivity per gram body weight were given according to the notations in the figure.

Fig. 2 Incorporation of ^{3}H-thymidine and ^{3}H-uridine in the rat brain as a function of the injected amount of precursor. Each thymidine point (o) represents the mean value of measurements on the DNA extracts from four animals while each uridine point (o) represents measurement on the RNA extract from one animal.

Fig. 3 Body weight for normal and irradiated rats during the first month after birth. Irradiation was always done within one day after birth. Each point represents measurements on 15-20 animals divided in two, three or four litters.

Fig. 4 Weights of the MMM and the TD parts of the brain for normal and irradiated rats as a function of time. Each point represents measurements on 14 animals divided in two litters.

Fig. 5 DNA (a) and RNA (b) contents of the whole brain in normal developing rats.

Fig. 6 DNA and RNA content of both the TD and MMM parts of the brain for normal and irradiated rats. Irradiation was performed within one day after birth, which is indicated with broken arrows. Each point represents measurements on 14 animals divided in two litters.

Fig. 7 Counted activity of ^{3}H-thymidine normalized to both the DNA content and weight for the TD and the MMM part of the brain. Each point represents measurements on one litter containing eight animals.

Fig. 8 Counted activity of ^{3}H-uridine normalized to both RNA content and weight for the TD and the MWM part of the brain. Each point represents measurements on one litter containing six animals.

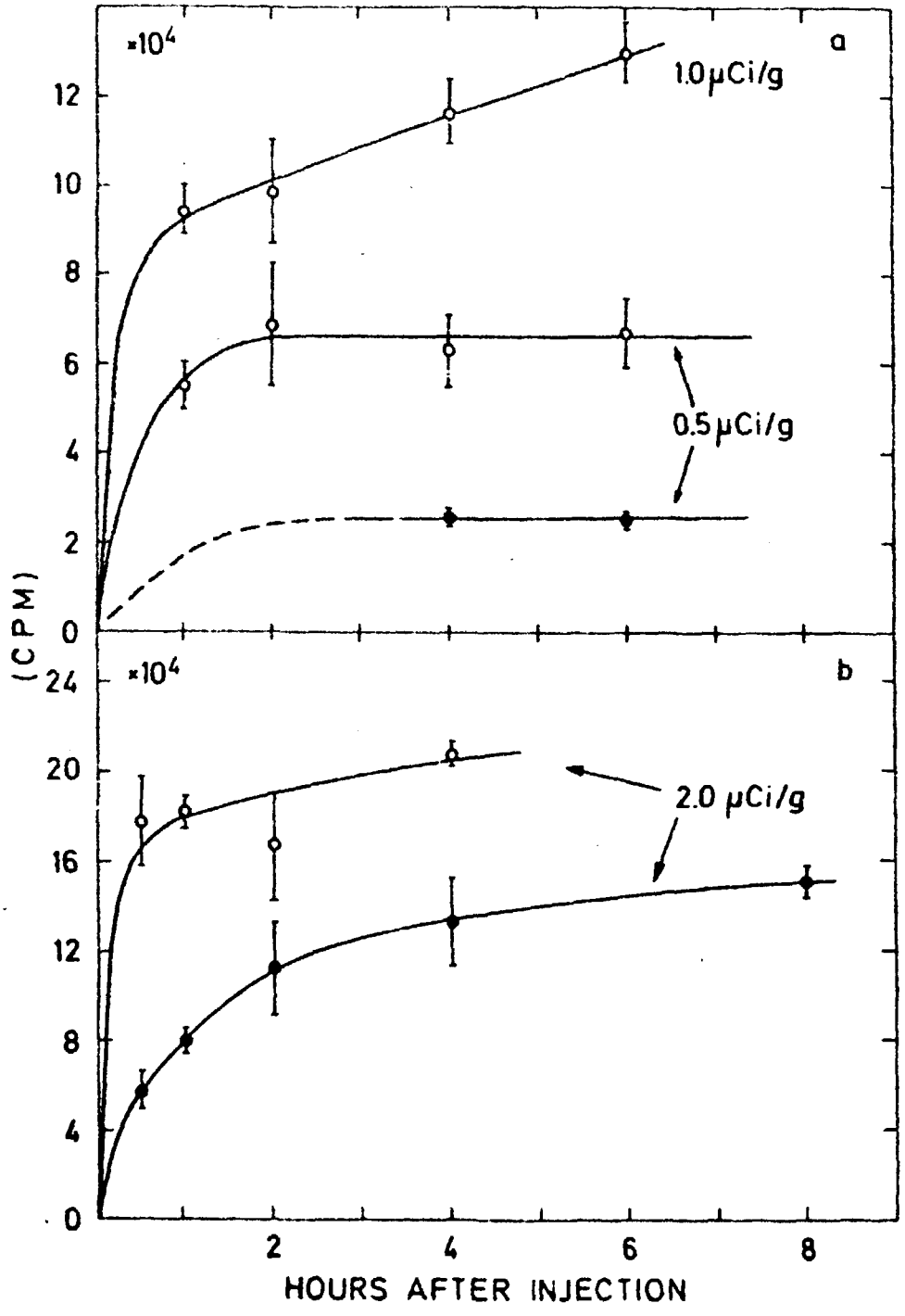


Fig 1.

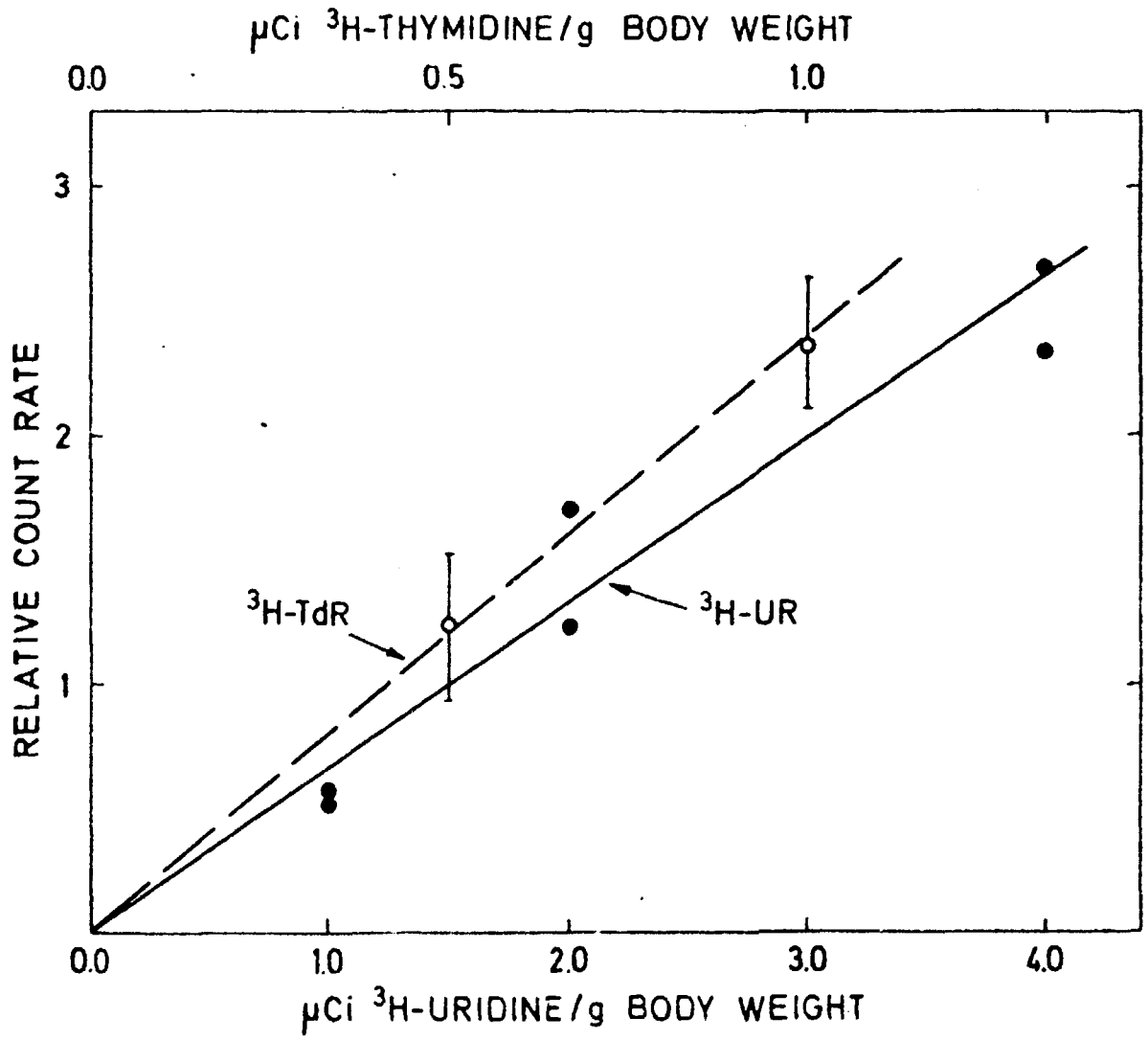
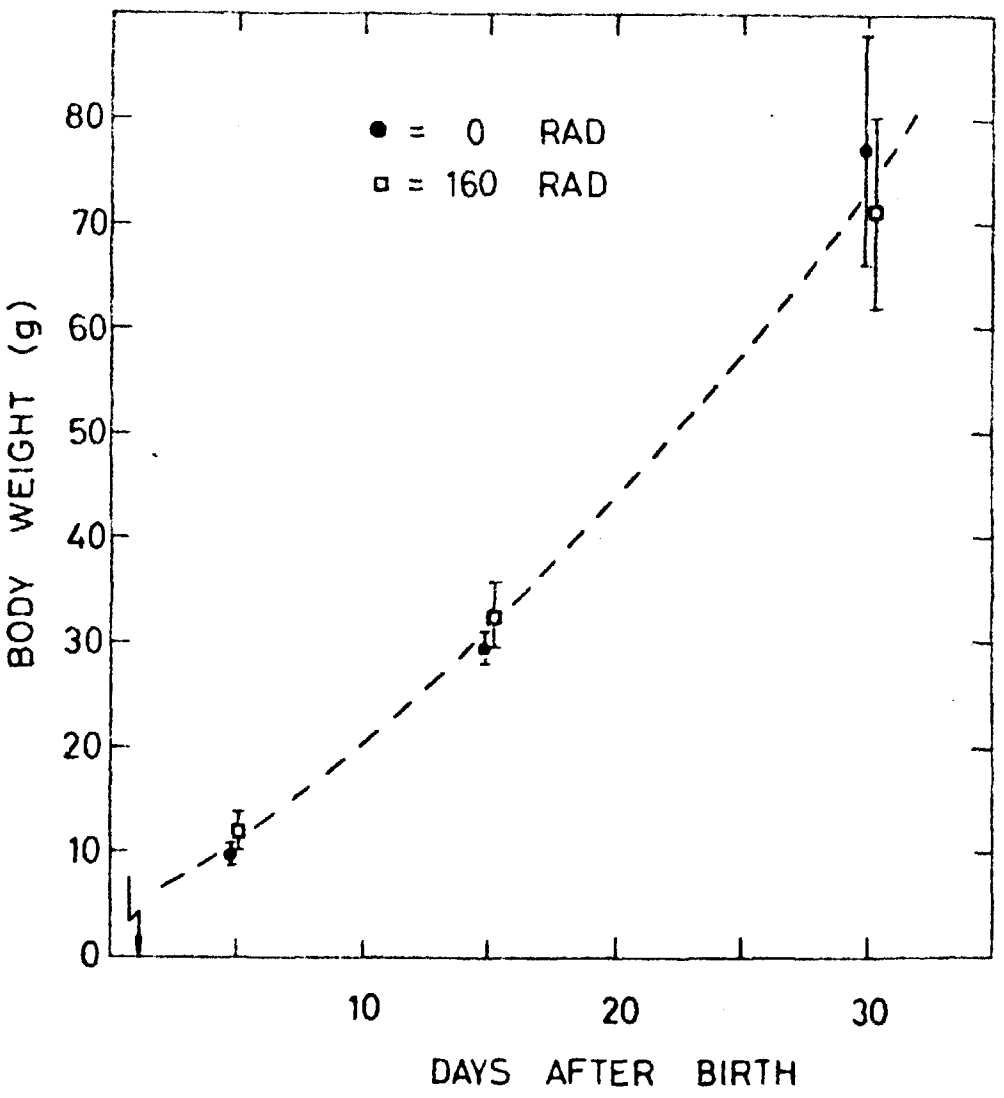

μCi ³H-THYMIDINE/g BODY WEIGHT
0.0
0.5
1.0
3
2
1
RELATIVE COUNT RATE
³H-TdR
³H-UR
0.0
1.0
2.0
3.0
4.0
μCi ³H-URIDINE/g BODY WEIGHT

Fig 2.


● = 0 RAD
□ = 160 RAD
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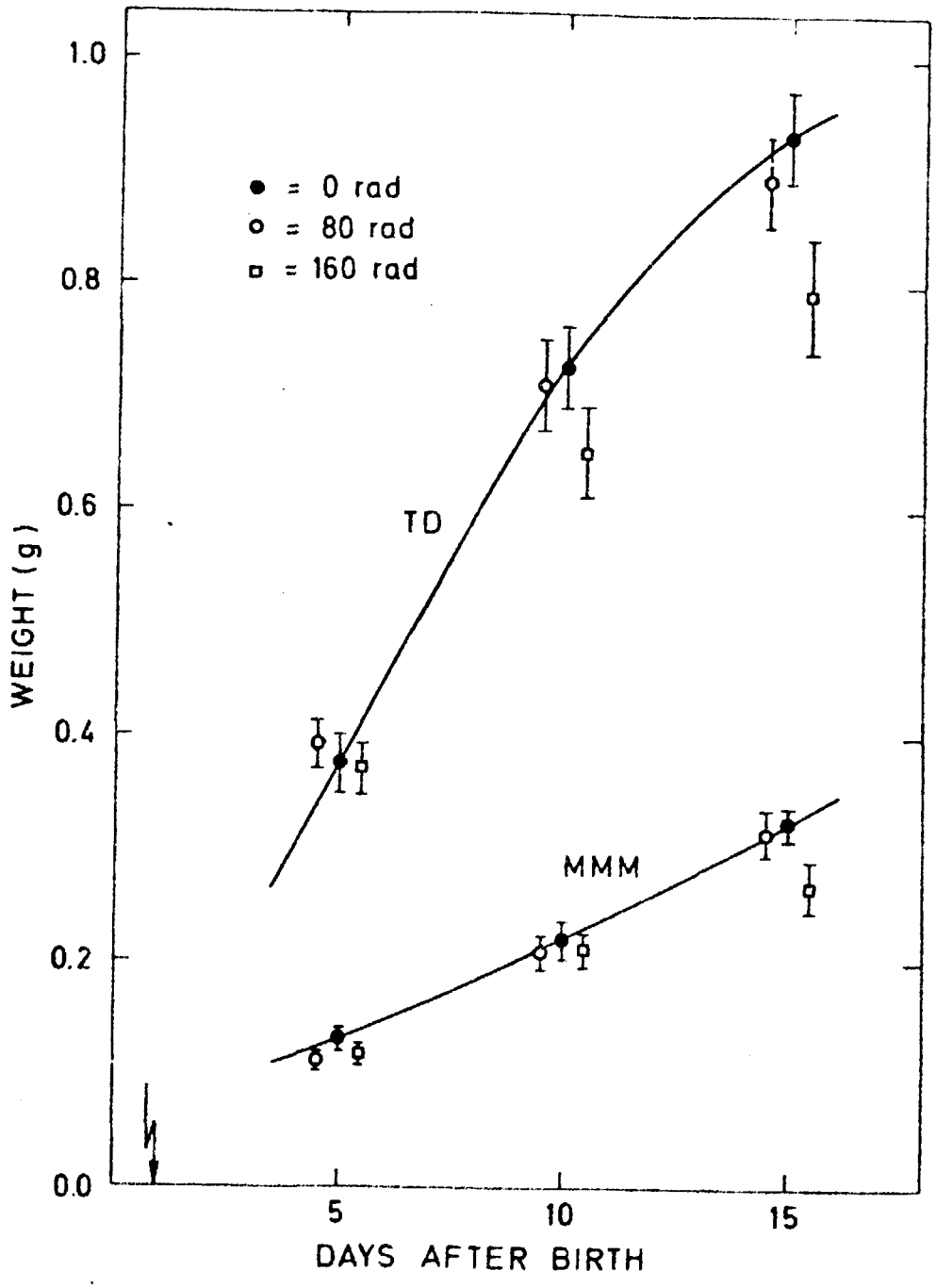
10
20
30
40
50
60
70
80
10
20
30
DAYS AFTER BIRTH

Fig 3,


1.0
0.8
0.6
0.4
0.2
0.0
WEIGHT (g)
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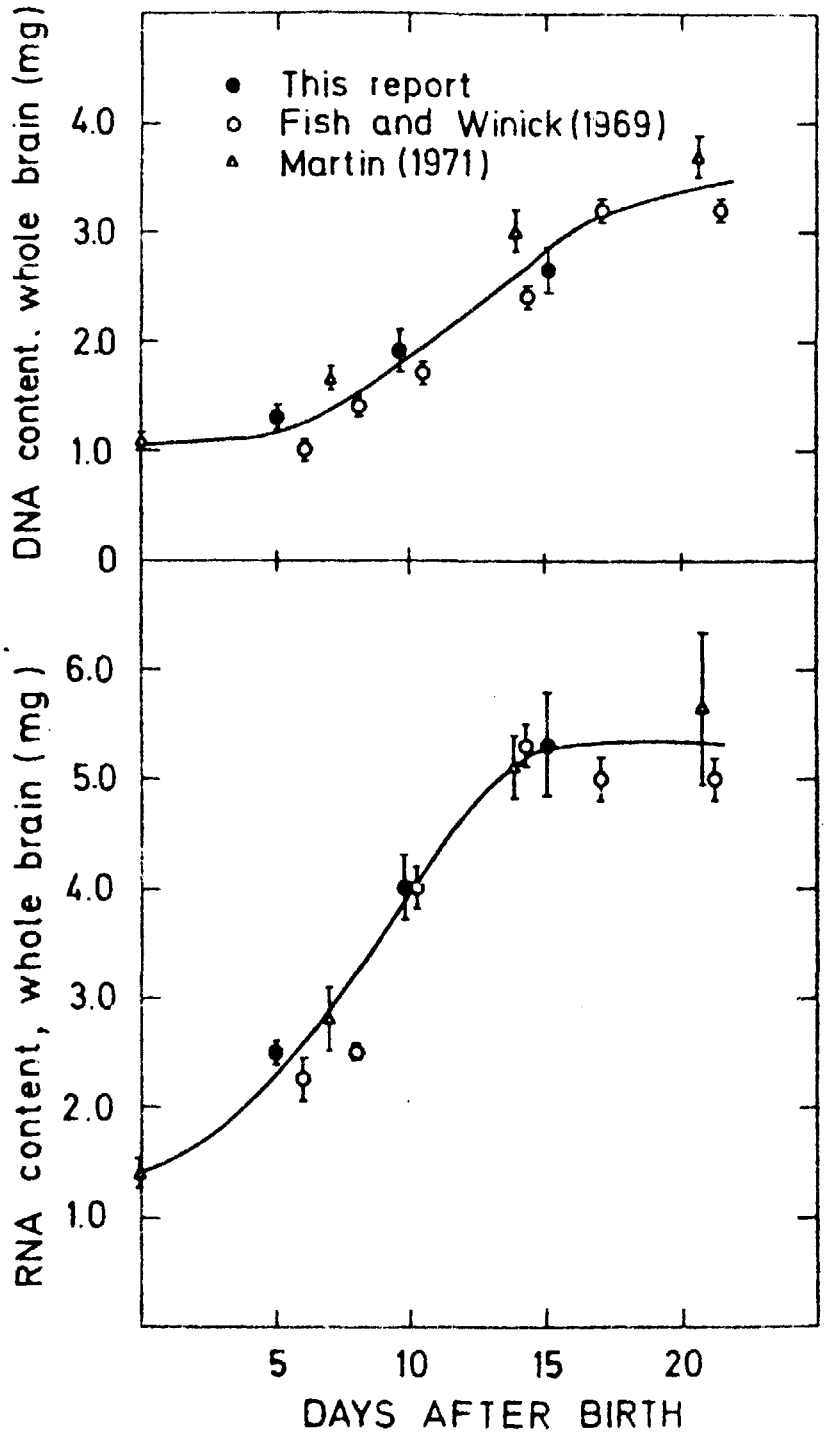
○ = 80 rad
□ = 160 rad
TD
MMM
5
10
15
DAYS AFTER BIRTH

Fig 4.


This report
Fish and Winick (1969)
Martin (1971)
DNA content, whole brain (mg)
4.0
3.0
2.0
1.0
0
RNA content, whole brain (mg)
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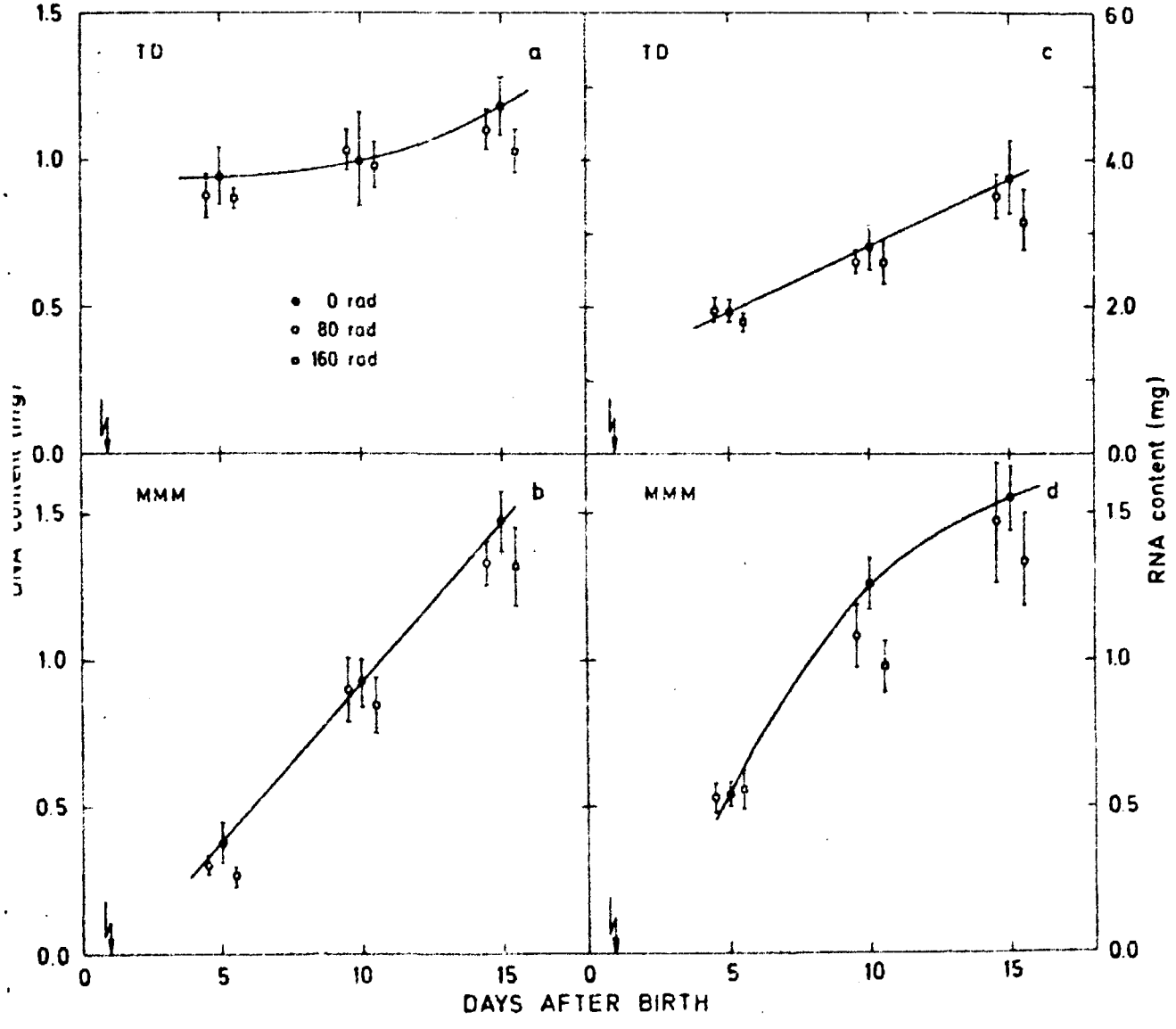
5.0
4.0
3.0
2.0
1.0
5
10
15
20
DAYS AFTER BIRTH

Fig 5.


TD
a
TD
c
MMM
b
MMM
d
0 rad
80 rad
160 rad
RNA content (mg)
DAYS AFTER BIRTH
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5
10
15
0.0
0.5
1.0
1.5
2.0
4.0
6.0

Fig 6.

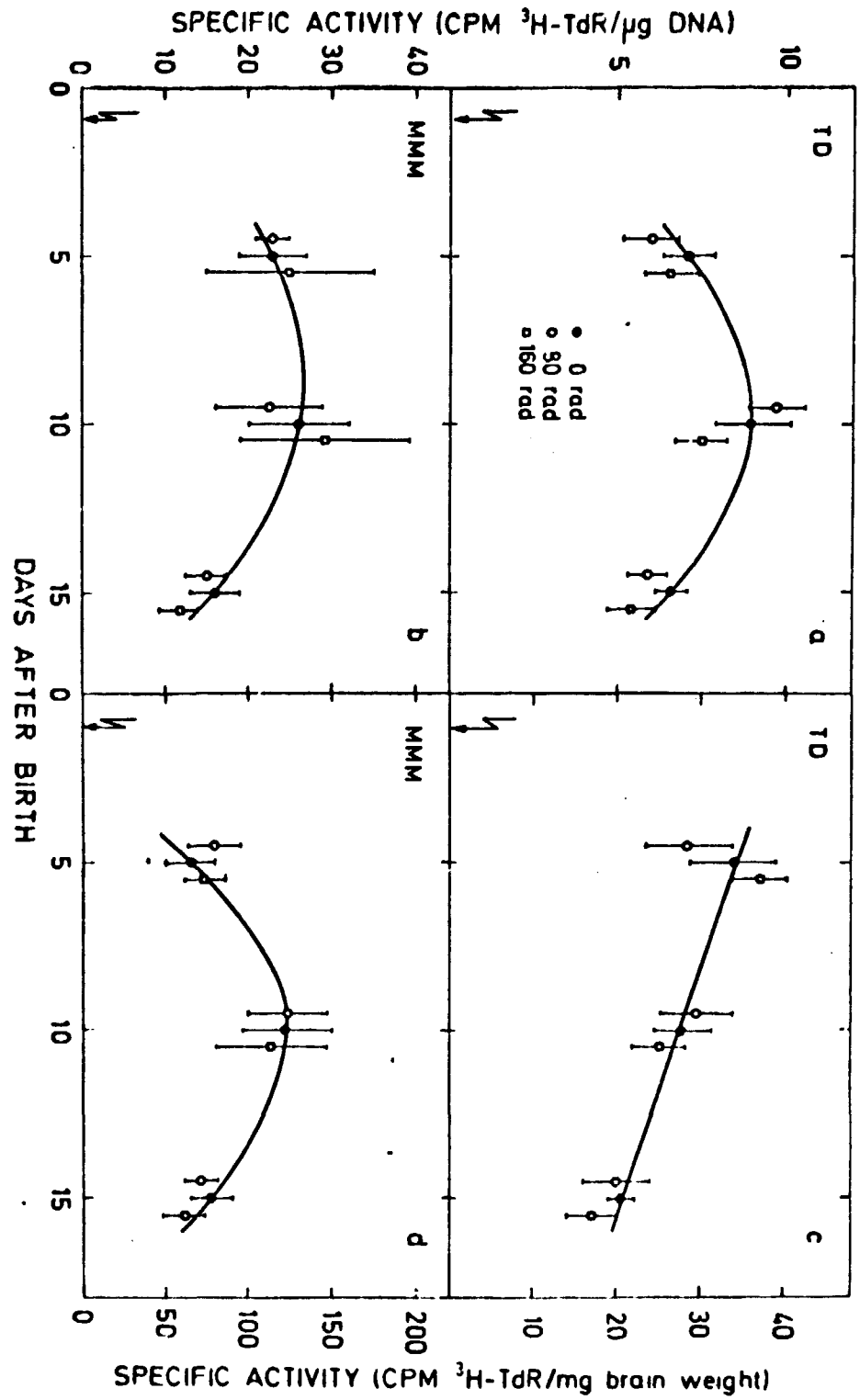
SPECIFIC ACTIVITY (CPM ³H-TdR/μg DNA)
SPECIFIC ACTIVITY (CPM ³H-TdR/mg brain weight)
DAYS AFTER BIRTH
MMM
TD
a
b
c
d
0 rad
90 rad
160 rad

Fig.7

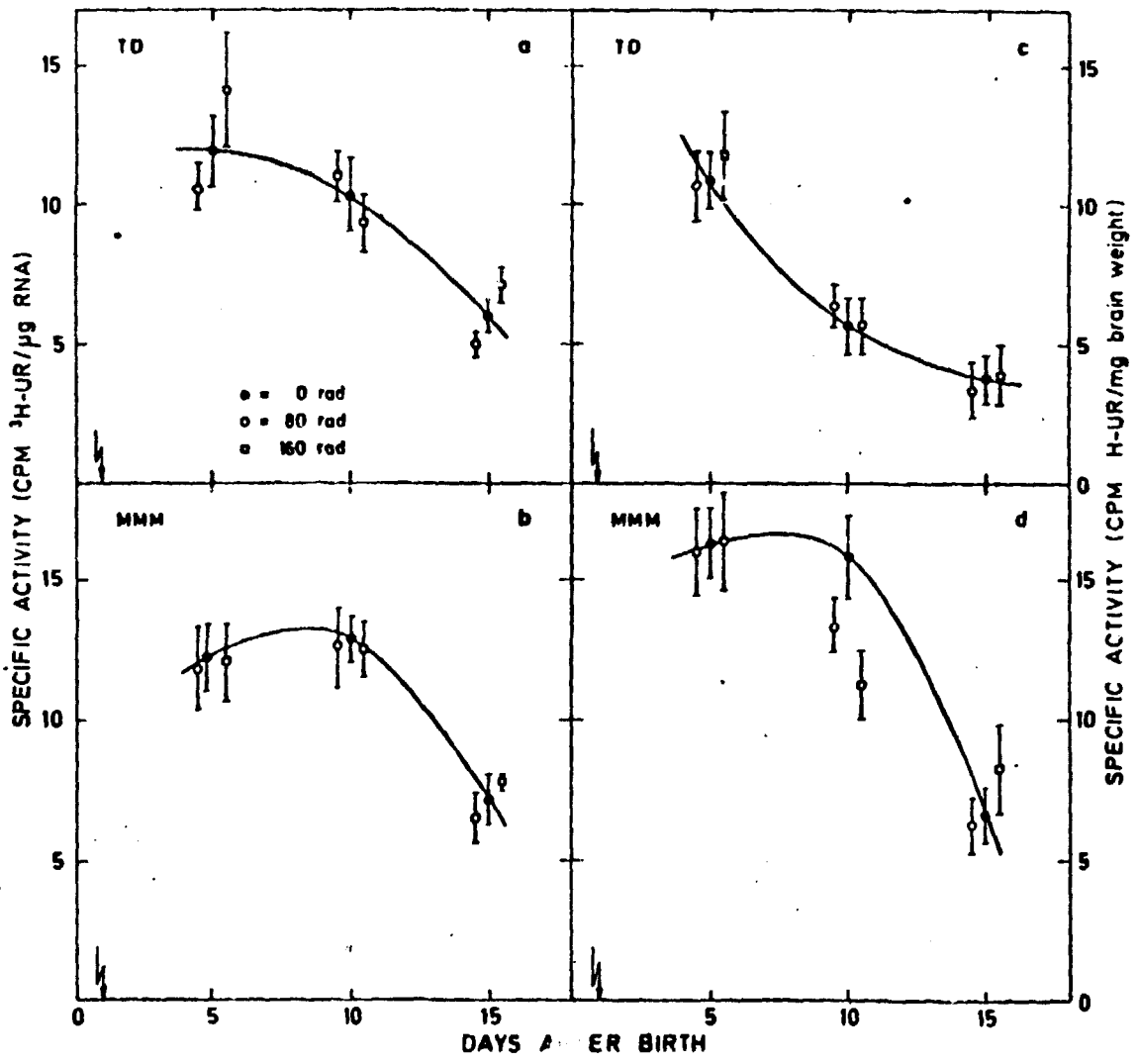
TD
a
TD
c
MMM
b
MMM
d
● = 0 rad
○ = 80 rad
▫ = 160 rad
SPECIFIC ACTIVITY (CPM ³H-UR/µg RNA)
SPECIFIC ACTIVITY (CPM H-UR/mg brain weight)
DAYS AFTER BIRTH
0
5
10
15

Fig.8