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Experimental Work on Induced Mutations [and Discussion]

Mary F. Lyon, H. J. Evans, A. G. Searle, J. H. Edwards and H. Sharma

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Experimental work on induced mutations

By Mary F. Lyon, F.R.S.
M.R.C. Radiobiology Unit, Chilton, Didcot, Oxfordshire OX11 0RD, U.K.

The detection of changes in mutation rate in human populations remains extremely difficult. Thus estimation of genetic hazards of mutagens to man depends on extrapolation from experimental systems. Germ cells of animals show complex variations in sensitivity to mutagenic effects. Some agents predominantly affect stem cells or other immature germ cells, whereas others mainly affect later germ cell stages. Dose–response relations also vary both with the agent and with the stage or sex of germ cell treated. In man, in addition to single-gene defects and chromosome anomalies, conditions of complex or uncertain inheritance, such as congenital malformations, are clinically important. Genetic theory leaves unclear whether the incidence of these would be affected by a change in mutation rate. Recent research has shown that in mice the incidence of malformations is increased by exposure of the parents to mutagens, but the effect is small. Chromosomal non-disjunction is also clinically important. Again, recent research shows that its frequency can be changed by mutagens, but the effects vary with germ-cell stage. Thus, further research is needed to elucidate the relative contributions of different environmental mutagens to human genetic disease.

Introduction

In man it is relatively simple to detect mutation in somatic cells, but much more difficult to study germ cells. So far, no clear evidence has been obtained of increased genetic disease due to raised mutation rates in human populations (Schull *et al* 1981; Mulvihill 1982; Lyon 1985). Therefore, we must rely on experimental evidence to predict what damage may be caused by mutagens in human germ cells.

The most important point to emerge from this evidence to date is that the responses occurring in germ cells are highly complex. At present there is no way of predicting effects in human germ cells, either from work on somatic cells or from studies of germ cells of lower organisms, such as insects or worms (Lyon 1982, 1983). The factors leading to these problems include wide variations in sensitivity of different sexes and developmental stages of germ cells (Lyon 1981), the complexity of the cell populations, and the DNA repair phenomena involved. Some of the agents potentially affecting mutation rate that must be considered include radiation, chemicals, transposable elements in the genome, and biological factors such as parental age.

Radiation

By far the most data are available for ionizing radiation (Sankaranarayanan 1982). In males, the germ-cell stages of most interest are the spermatogonial stem cells, because they accumulate an environmental radiation dose throughout the life of the individual. These cells show an example of the problem of complex cell populations. The mutagenic response to an acute dose of radiation to these cells is humped, both for the endpoint of gene mutations (Russell & Russell 1959) and for chromosome translocations. The dose–response curve for

induction of chromosome translocations by X-rays in mice (Savkovic & Lyon 1970) reaches a peak at 600–700 cGy and then falls (figure 1). The interpretation is that the cell population is heterogeneous in sensitivity, both to mutagenesis and to cell-killing. As the dose is increased the sensitive cells are preferentially killed, leaving only the mutagenic response of the resistant fraction (Leenhouts & Chadwick 1981; Cattanach 1986). Similar dose–response curves have been obtained with other mammalian species, including laboratory species and various primates, up to about nine species so far (Lyon & Cox 1975; van Buul 1980, 1983; Matsuda *et al.* 1984, 1985). All species show a similar shaped curve, but all have the peak at a lower dose than in the mouse (figure 2). There are even a few data from man, suggesting that the peak response of the human testis is at only about 200 cGy (Brewen *et al.* 1975). Thus it would be fruitless to study humans who had received high doses to the testis to predict effects at lower doses. In considering human hazards it is of course the effects of small doses and low dose-rates that are of major relevance.

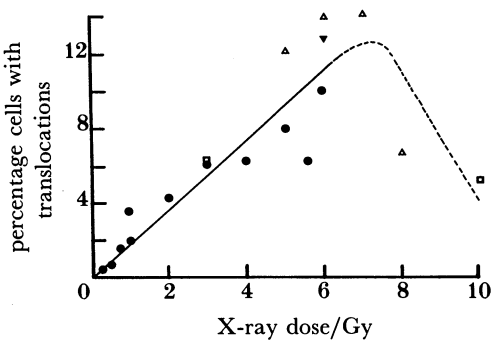

FIGURE 1. Dose–response curve for the yield of translocations detected in mouse spermatocytes after irradiation of spermatogonial stem cells. (Reprinted with permission from Savkovic & Lyon (1970).)

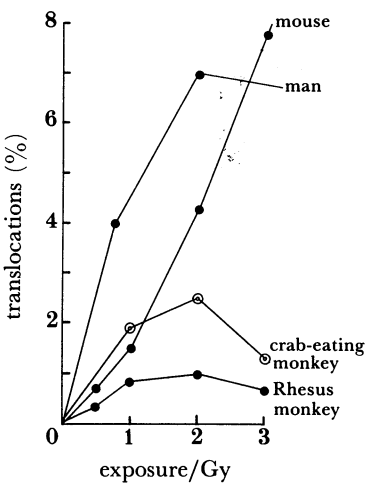

FIGURE 2. Dose–response curves for the yield of translocations detected in spermatocytes after irradiation of spermatogonia of mouse and some primate species. (Reprinted with permission from Matsuda *et al.* (1984).)

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The dose–response relation for specific-locus gene mutation in female mice shows another phenomenon. The response obtained after X-ray doses to mature oocytes is markedly curved (Lyon *et al.* 1979), with a higher mutation rate per unit dose after higher doses (table 1). As gene mutations are single-hit events, a linear response might have been expected. The interpretation of the curved response has been the subject of controversy (Abrahamson & Wolff 1976; Russell 1977). The most probable explanation appears to be that the effect is attributable to DNA repair, which becomes saturated at higher doses thus giving the upward curve to the response.

TABLE 1. SPECIFIC LOCUS MUTATIONS AFTER X-IRRADIATION OF MATURE MOUSE OOCYTES

(Data from Lyon *et al.* (1979).)

dose / cGy	mutants	total offspring	$10^7 \times$ mutation rate / locus cGy^{-1}
200	7	18867	2.65
400	7	7501	3.30
600	26	9875	6.27

TABLE 2. SPECIFIC LOCUS MUTATIONS AFTER SINGLE AND FRACTIONATED DOSES OF X-RAYS TO MOUSE OOCYTES

(Data from Lyon & Phillips (1975).)

dose / cGy	mutants	total offspring	$10^7 \times$ mutation rate / locus cGy^{-1}
1×200	7	21578	2.32
20×10	1	20398	0.35

As might be expected from the curved response to single exposures, both division of the dose into many small fractions (Lyon & Phillips 1975) (table 2) and a reduction of the dose-rate (Russell 1965) give a reduction in response. Similar, though less pronounced, reduced effects of repeated small doses (Lyon *et al.* 1972) or of low dose-rates (Lyon *et al.* 1975) are obtained with spermatogonial stem cells (figure 3). Here the explanation is probably more complex, as the repeated or prolonged radiation exposures are likely to change the cell population characteristics, in addition to having effects on repair. Thus, in germ cells of either sex, small environmental radiation doses would be of relatively little effect.

Another phenomenon seen in female mice is the change in sensitivity of the oocyte at different stages of development. This changing sensitivity is shown by a change in response to a given radiation dose with increasing interval between irradiation and mating. Kirk & Lyon (1982) irradiated female mice with doses of 108–504 cGy X-rays, mated them at intervals ranging up to 4 weeks after irradiation and observed the incidence of congenital malformations in the progeny. The incidence increased both with dose and with time interval, the maximum effect being obtained in the third week after irradiation (table 3). This was in line with data on dominant lethal mutations obtained in the same experiment, and with earlier work by others on both dominant lethals and specific locus mutations (Russell 1977; Searle & Beechey 1974). Thus, the relatively immature growing oocytes sampled in week 3 were more sensitive than the mature oocytes in week 1.

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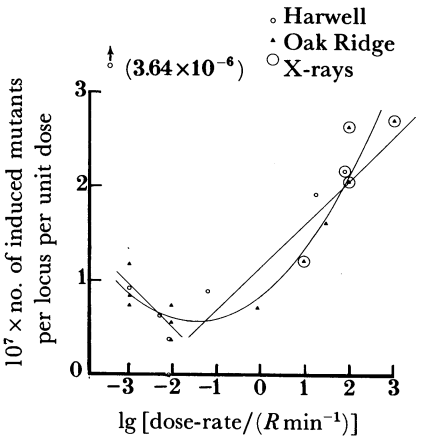

FIGURE 3. Relation of specific locus mutation per locus per unit dose (R) to dose-rate, after irradiation of mouse spermatogonial stem cells. (Modified from Lyon *et al.* (1972).)

TABLE 3. INCIDENCE OF ABNORMAL FETUSES FROM IRRADIATED FEMALES

(Results expressed as percentage ratio abnormal: live fetuses; reprinted, with permission, from Kirk & Lyon (1982).)

	absorbed dose/cGy			
interval/d	108	216	360	504
1–7	1.8±0.9	0	1.8±0.8	2.8±1.3
8–14	0.8±0.7	3.6±1.3	5.6±2.0	5.4±1.9
15–21	1.5±1.0	4.3±2.5	8.4±5.5	12.5±3.2
22–28	1.2±1.1	4.6±5.8	3.3±1.9	11.8±4.0
Control		1.1±0.4		

The work on congenital malformations is also relevant to the assessment of human hazards. In genetic epidemiology, the incidence of malformations is sometimes used as a potential indicator of an increase in mutation rate (Mulvihill & Byrne 1985). However, in view of the complex inheritance of these anomalies, including possible environmental effects, genetic theory leaves doubt as to whether the incidence would in fact be affected by mutation. Kirk & Lyon (1984) studied the incidence of malformations in the offspring of male mice, after treatment of spermatogonial stem cells. If the size of the effect is compared with the spontaneous incidence it is possible to calculate the dose required to double the incidence (table 4). For radiation delivered at low dose-rates or in small fractions this dose is about 650 cGy, more than six times the doubling dose usually accepted for defects due to single genes (Sankaranarayanan 1982). This high doubling dose is as would be expected from the complex inheritance of malformations. Thus, congenital malformations provide a very insensitive indicator of change in the mutation rate.

Other evidence relevant to human hazards, particularly involving female germs cells, comes from work of Tease (1982) on varying sensitivity of stages of oocyte development to chromosomal non-disjunction. From epidemiological studies there was controversy as to whether environmental radiation increased the incidence of Down's syndrome in man. Tease (1982) showed that, in female mice, irradiation with doses of 10–50 cGy at the highly sensitive stage just before ovulation gave a linear dose-related increase in aneuploidy (figure 4).

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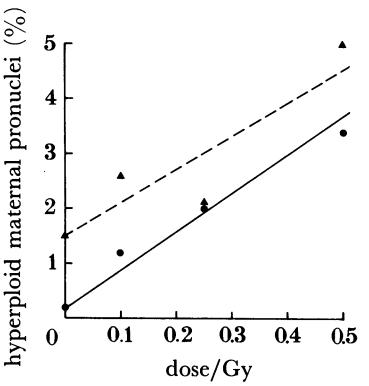

FIGURE 4. Dose–response relations for the induction of hyperploidy by irradiation of immediately preovulatory oocytes in young (solid line) or old (broken line) female mice. (Reprinted, with permission, from Tease (1982).)

TABLE 4. INCIDENCE OF ABNORMAL FETUSES AFTER IRRADIATION OF STEM-CELL SPERMATOGONIA, AND DOUBLING DOSE

(Data from Kirk & Lyon (1984).)

X-ray dose / cGy	abnormal fetuses (%)	induced (%)	induced per cGy
0	0.7 ± 0.3	—	
500	2.2 ± 0.5	1.5	3.0×10^{-5}
500 + 500	3.1 ± 0.6	2.4	2.4×10^{-5}

spontaneous $= 0.7 \times 10^{-2}$

mean induced per cGy $= 2.7 \times 10^{-5}$

doubling dose $= \dfrac{0.7 \times 10^{-2}}{2.7 \times 10^{-5}}$

$= 259$ cGy.

applying correction factor (2.5×) for low dose-rate; doubling dose $= 648$ cGy.

However, when irradiation was during weeks 1–4 before mating, when the oocytes were again at a fairly sensitive, but not highly sensitive, stage, doses of 100–600 cGy were needed to give a measureable induction (Tease 1985), this time with a curved dose-response (figure 5) as with gene mutation. Thus, this work has shown that radiation can indeed induce aneuploidy. However, the stages studied were not those of greatest interest in relation to human hazards. The oocyte receives the greater part of the lifetime dose of radiation while it is in the resting or primary oocyte stage. In the mouse this stage is highly sensitive to cell killing by radiation, and hence it is difficult to obtain data on its genetic sensitivity. It has been thought to be genetically insensitive (Russell 1977). Recently, however, Griffin & Tease (quoted in Tease (1987)) have found both aneuploidy and chromosome structural aberrations in oocytes irradiated with γ-rays at low dose-rate at the primary oocyte stage, and Dobson *et al.* (1987) have similarly found chromosome aberrations after treatment of mouse primary oocytes with ionizing

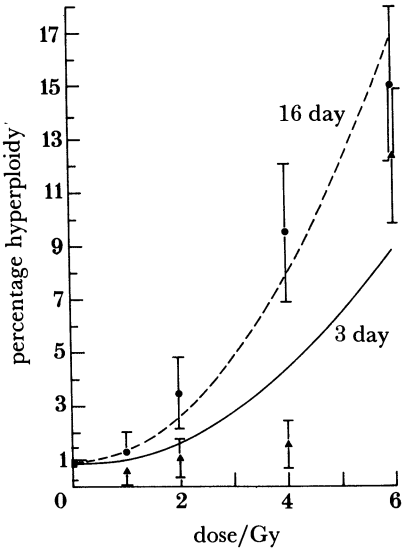

FIGURE 5. Dose–response relation for the induction of hyperploidy by irradiation of dictyate mouse oocytes in week 1 (3 day) and week 3 (16 day) before mating. (Based on data of Tease (1984).)

particles. In assessing human hazards, there had been controversy as to whether the human primary oocyte should be considered susceptible to radiation genetic damage (Russell 1977; BEIR 1980) in view of the apparent insensitivity of the corresponding mouse cell type. Now that genetic damage has been found in mouse primary oocytes, it seems clear that the human primary oocyte should be considered as potentially susceptible. Exactly how sensitive it may be is not clear. In view of the previously mentioned reduced effect of small doses and low dose-rates in mouse oocytes, however, the effect of environmental radiation doses is probably less and certainly not greater in the female than that in the male.

PARENTAL AGE

The work on aneuploidy has provided some evidence on the effect of parental age on mutation rate. In the mouse, as in the human, the spontaneous incidence of aneuploidy increases with maternal age. When young and old female mice were irradiated at the highly sensitive pre-ovular stage, the regression lines of increase of aneuploidy with dose ran parallel to each other (Tease 1982) (figure 4). Thus, there was no evidence that the higher incidence in old mothers was due to increased sensitivity to mutagens. On the other hand, Cattanach (1966), using the mutagenic chemical triethylene melamine (TEM) and treating male mice, found that animals treated when a year old seemed about five times as sensitive as young adult (3–4 months) mice to the induction of specific locus mutation. Hence, the possibility of any change in sensitivity to mutagens with age obviously needs further work.

CHEMICAL MUTAGENS

Concerning chemical mutagens in general, the most striking phenomenon is the very marked variation in sensitivity of different germ-cell stages with different mutagens (Lyon 1981). If we

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Table 5. Relative mutagenic sensitivity of mouse male germ-cell stages

mutagenic agent	stem cells	postmeiotic cells
Radiation	+	++
TEM	+	+++
procarbazine	+	+
ENU	+++	+
EMS	–	+

consider data from male mice only, all possible types of pattern of sensitivity of different stages of spermatogenesis are seen (table 5). Some agents, such as ethylnitrosourea (ENU), act predominantly on spermatogonial stem cells (Russell 1982). Others, such as TEM and ethylmethane sulphonate (EMS), affect mainly postmeiotic stages; procarbazine appears to affect all stages more or less equally (Lyon 1981). The ratios of the sensitivities of the various stages may be very large. With ENU, stem-cells are about ten times as sensitive as postmeiotic cells (Russell 1982). With TEM, conversely, postmeiotic stages are 300 times as sensitive as stem cells to gene mutation and 1000 times as sensitive to chromosome aberrations (Lyon 1981). At present the basis of these variations in sensitivity is not understood, and more fundamental knowledge on DNA lesions induced by chemicals and their repair in mammalian germ-cells is badly needed.

Transposable elements

Finally, there is the question of mutagenesis due to transposable elements in the genome. In the fly, *Drosophila melanogaster*, a large fraction of all spontaneous mutations are thought to be associated with transposable elements (Rubin 1983). It is known that inserted retroviral and other sequences can cause mutation in the mouse, from the results of work in which foreign DNA sequences have been experimentally inserted into the genome. Some examples of the ensuing mutations include a recessive visible mutation, causing limb deformity (Woychik *et al.* 1985), mutations to HPRT deficiency (Kuehn *et al.* 1987), and a recessive lethal mutation due to a collagen deficiency (Schnieke *et al.* 1983). However, there is less evidence that spontaneous mutations in mice involve transposable elements. Jenkins *et al.* (1981) and Copeland *et al.* (1983) found that the coat-colour mutation 'dilute' was associated with a proviral insertion, and that excision of the viral insertion led to reverse mutation to the wild type. However, this is the only spontaneous mutation so far found to be associated with a transposable element. Other spontaneous mutations analysed have included intragenic deletions, involving haemoglobin (Skow *et al.* 1983), myelin basic protein (Molineaux *et al.* 1986), and gonadotrophin-releasing hormone genes (Mason *et al.* 1986), and a multi-locus deletion on chromosome 17, called hairpin-tail (Bennnett 1975). Spontaneous mutations in the H-2 complex have been attributed to gene conversion events (Hansen *et al.* 1984). Thus, the evidence so far suggests that transposable elements may be of less importance in spontaneous mutation in the mouse than in *Drosophila*. Sankaranarayanan (1986) arrived at a similar conclusion for man. There is preliminary evidence that mutagens can cause excision of inserted elements in the mouse, as in other organisms, in that X-rays induced reverse mutation at the dilute locus (Favor *et al.* 1987), but again much more work is needed.

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Conclusions

Thus, experimental work has provided some answers and left various unsolved problems concerning germ-cell mutation in mammals. Ionizing radiation and some chemicals are both clearly mutagenic. At the low doses and dose-rates at which environmental radiation is received it can account for only a small fraction of the mutation that is occurring. It is very difficult to assess the importance of the mutagenic effect of environmental chemicals, because of the very wide variations in sensitivity of germ-cells. The major part of the data available concern males, and the majority of the chemicals tested so far fall in the category of those that have little effect on spermatogonial stem cells (Russell 1986). Thus, for these it is only any dose received in a few weeks or months before conception that need be considered. This might be important in the case of occupational accidents, or where a mutagenic chemical had been given as part of medical treatment. For example, some anti-cancer drugs and immunosuppressive drugs are mutagenic (Mulvihill 1982; Lyon 1985). However, probably very few conceptions occur in such circumstances. In relation to effects of parental age on sensitivity to mutagens, and on the possible role of insertions of transposable elements into the genome we need further work. We badly need new and less costly and laborious methods of studying mutagenesis and the molecular basis of mutation in germ cells, and it is to be hoped that the new DNA technology will soon provide such methods.

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Discussion

H. J. Evans (*Medical Research Council, Edinburgh, U.K.*). In man there is evidence for recombinational hot spots for somatic cell translocations, as for example in relation to the activation of certain *Onc* genes, such as *c-abl*, where *Alu* middle repeat sequences provide sites for homologous sequence exchange between non-homologous chromosomes. Is there any evidence in the mouse for a localization of structural rearrangements to sequences belonging to the B-middle repeats, which would parallel the finding in man, and is there other evidence for mutational hot spots in the mouse genome?

Mary F. Lyon, F.R.S. At present there is little evidence for the presence of mutational hot spots in the mouse comparable to those found in man. It is possible that this lack of evidence is simply due to less intensive study of the phenomenon. A recombinational hot spot has been found in the H-2 complex in the mouse (Steinmetz *et al.* 1986). In addition, in the H-2 complex, the t-complex on chromosome 17, and the haemoglobin complex, it is thought that mutations can arise from misalignment of repeated genes with highly homologous sequences. However, as far as I know, the role of the B elements in mutation in the mouse is still unknown.

Reference

Steinmetz, M., Stephan, D. & Fischer-Lindahl, K. 1986 Gene organization and recombinational hotspots in the murine major histocompatibility complex. *Cell* **44**, 895–904.

A. G. Searle (*Medical Research Council, Chilton, U.K.*). Dr Lyon mentioned recent findings that show that chromosome aberrations can be induced in immature mouse oocytes. However, in view of previous work at Oak Ridge in which no specific locus mutations could be recovered after irradiation of this stage, does she not think it would be worthwhile to carry out more investigations of this kind on other experimental mammals with immature oocytes more similar to human ones?

Mary F. Lyon, F.R.S. It certainly would be very valuable to have more data on mutagenic response of oocytes in other mammalian species. The primary oocytes of the guinea pig are much more resistant to killing by radiation than those of the mouse. The evidence so far on the genetic sensitivity of these cells is that dominant lethal mutations are induced in them, and can be detected in animals mated several months after exposure to radiation. However, the sensitivity is fairly low, and less than that of mature oocytes. More information on the genetic sensitivity of primary oocytes of various mammalian species would be very valuable for extrapolation to the human.

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J. H. Edwards, F.R.S. (*Genetics Laboratory, University of Oxford, U.K.*). Dr Lyon mentioned insertional elements as a possibly substantial cause of mutational events in mammals: would she expect such events to be commoner after exposure to radiation or other common mutagens?

Mary F. Lyon, F.R.S. As I mentioned, there is preliminary evidence that radiation can increase the rate of excision of inserted elements in the mouse, from the work of Favor *et al.* (1987) on reverse mutation at the dilute locus. In *Drosophila melanogaster* the evidence is equivocal; some studies suggest that mutagens do indeed increase the rate of movement of insertional elements, and other studies give negative results (reviewed by Sankaranarayanan (1986)). Clearly, further work is needed. In both *Drosophila* and mouse, certain strains are 'permissive' for movement of elements, so that the offspring of crosses of these permissive strains to those carrying movable elements carry many new insertions. The relevance of this phenomenon to mutation in man is not yet clear.

H. Sharma (71 *Barrack Road, Hounslow, U.K.*). Can Dr Lyon discuss the sensitivity to mutagenic and cytotoxic effects of an agent in pre-pubertal and sexually mature humans?

Mary F. Lyon, F.R.S. Research has been carried out on the genetic sensitivity to radiation of fetal and prepubertal mouse germ cells. The cells go through complex changes in sensitivity as development proceeds. In the male, the germ cells of prepubertal animals are in general less sensitive to mutagenesis than those of adults. In the female there are certain stages that are more sensitive to mutagenesis, and other stages more sensitive to cell killing than those of the adult. Extrapolation of these findings to man gives no reason to think that environmental radiation doses to young individuals pose any much greater hazard than that to adults.

H. Sharma. Can Dr Lyon discuss differences in human male and female gamete production and effects on mutagenic and cytotoxic effects of an agent?

Mary F. Lyon, F.R.S. Basically, the processes of gamete production in the human are similar to those in other mammals. In males, when the variations in sensitivity of different stages of development of germ cells have been compared among species, the general patterns of variation have been the same for all species. However, there have been quantitative differences. Thus, in extrapolating from animals to man, the general principles probably hold good, but it is not known how great the quantitative differences may be. Among females, less work on species comparisons has been done. In most species studied, including the human, primary oocytes are less sensitive to cell killing by radiation than those of the mouse. More work is needed on the genetic sensitivities of oocytes in different species. In guinea pigs such cells can be mutated by radiation, but with low sensitivity. Thus, we must assume that human primary oocytes can be affected by mutagens, but as with males, it is very difficult to make any quantitative predictions.

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