

# FURTHER STUDY OF INDUCTION OF BRAIN TUMORS IN RATS BY METHYLNITROSOUREA

I. N. Dimant, G. M. Loktionov,  
M. M. Sataev, and A. A. Israilyan

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The authors conclude from the analysis of their own observations and data in the literature that a significant connection exists between the incidence of brain tumors in rats and disturbances of the hormonal balance produced by x-ray irradiation and administration of methylnitrosourea.

Since the discovery that the carcinogenic action of certain nitrosoamines on nerve tissue is characterized by selectivity [7, 8], conflicting facts have been reported in the literature summarizing recent findings [5, 6, 9, 10, 12] with respect to the incidence and localization of tumors induced by these compounds in the nervous system. Since the writers' first attempts to reproduce these experiments on noninbred rats were unsuccessful, like corresponding experiments by other workers, it was next decided to administer the carcinogen (methylnitrosourea - MNU) to experimental animals with a preliminarily induced hormonal imbalance.

Since half the animals taking part in such experiments developed brain neoplasms in the course of a year, it was decided to publish the preliminary results of these investigations in order to concentrate attention on the fact that brain tumors have been induced under the experimental conditions used [1]. Naturally, since the experiment was incomplete, it was impossible to make a final analysis of its results, and this fact has been commented upon [6].

The final results of these experiments are described below.

## EXPERIMENTAL RESULTS

The experimental methods used were described previously [1]. The results are given in Table 1. Statistical analysis of the material, in order to reveal the influence of hormonal imbalance on the incidence of brain tumors in the experimental groups of animals compared with the control, was carried out by the method of analysis of empirical distributions, by calculating the criterion of correspondence  $\chi^2$  most adequate for such cases, with the introduction of Yates's correction; a parallel determination of the measure of agreement was made by means of the coefficient of correlation, with an assessment of its significance [3, 4]. Values of the probabilities of the  $\chi^2$  criterion for the groups compared are summarized in Table 2.

As the results given in Table 2 show, comparison of the experimental group of females (1) with the control (3) and the combined control (3 + 4) revealed no statistically significant association between the incidence of brain tumors and hormonal imbalance due to x-ray irradiation of the ovaries.

In all of the other experimental groups compared, hormonal imbalance had a definite effect on the incidence of brain neoplasms induced by MNU; an average degree of correlation is found between the incidence of tumors and hormonal disturbances ( $r = 0.35-0.5$ ,  $P < 0.05$ ).

Histological examination of the ovaries from the animals of group 1 showed the development of follicular cysts (and, consequently, of hormonal disturbances in the form of endogenous hyperestrogenization)

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TABLE 1. Results of Induction of Brain Tumors in Rats by Intravenous Injection of MNU\*

Groups of animals	Experimental conditions	at beginning of experiment	Number of animals		
			at time of appearance of first tumor	with brain tumor	without brain tumor
1 (females)	X-ray irradiation on ovaries	10	9	5	4
2 (females)	Feeding with MNU	20	16	10	6
Total in experimental groups 1 and 2		30	25	15	10
3 (females)	Control 1	12	9	1	8
4 (males)	Control 2	8	8	2	6
Total in control groups 3 and 4		20	17	3	14

\*Results of a control group of the series carried out at the same time on 8 males are given in this table.

TABLE 2. Values of Criterion of Correlation ( $\chi^2$ ) and P Calculated from Tables of Contingency ( $2 \times 2$ )

Group of animals compared	Index		Note
	$\chi^2$	P	
1-3	2.2	> 0.10	Comparison of experimental groups with control group of females
2-3	4.26	< 0.05	
1 + 2-3	4.5	< 0.05	
1-3 + 4	2.3	> 0.10	Comparison of experimental groups with combined control group (females + males)
2-3 + 4	5.19	< 0.05	
1 + 2-3 + 4	5.7	< 0.05	

in only 6 of the 9 rats. In 5 of these 6 rats, brain tumors also developed (according to Ird [2], after irradiation of the ovaries under similar conditions follicular cysts developed in 65% of cases). If the value of the coefficient  $\chi^2$  is calculated for this relationship, in this group its value is 6.34 ( $P < 0.02$ ), and the coefficient of correlation  $r = 0.65$  ( $P < 0.05$ ). In this series of the experiment also, a definite association was thus established between the hormonal imbalance and induction of brain neoplasms by MNU.

Another fact which deserves equal attention is that, according to data in the literature, the incidence of brain neoplasms in rats following administration of MNU (in roughly identical experimental conditions) was largely dependent on the line of experimental animals used. For instance, Druckrey et al. [8], using line BDII rats, induced brain neoplasms in 30 of 37 animals (in 7 of 7 in series I, in 9 of 12 in series II, and in 14 of 16 in series III), while Jänisch et al. [6, 10], using Wistar rats, obtained tumors of the nervous system in 7 of 18 rats (2 brain tumors, 5 neuromas) and in 20 of 30 rats of line E.

Comparison of the incidence of tumors of the central nervous system in animals of line BDII and Wistar, and line E and Wistar revealed a definite dependence on the line of rats used (the values of  $\chi^2$  were 8 ( $P < 0.01$ ) and 11 ( $P < 0.01$ ), respectively]. This fact is confirmed by results obtained by other workers giving MNU by mouth: Druckrey et al. [7] obtained no brain tumors in BDII rats, Schreiber et al. [11] induced brain neoplasms in 6 of 34 line E and Wistar rats, while Thomas et al. [13] induced tumors in 16 or 20 Wistar rats.

The facts described above support the view that even the biological properties distinguishing individual lines of experimental animals definitely exert an effect on the final result of the carcinogenic action of alkyl derivatives of nitrosourea on the tissues of the central nervous system. Clearly, therefore, a disturbance of the hormonal balance of experimental animals, which itself modifies their biological properties, must exert some action on this process. The fact that Enish and co-workers [5] obtained negative results in experiments on castrated rats does not contradict, but confirms this hypothesis, because in that case it is probable that the reproduced endocrine pathology differed in its character and severity. Analysis of the final results of this investigation, accompanied by careful statistical treatment, thus confirms the views expressed in the preliminary report concerning the role of various predisposing factors (including hormonal) in the genesis of brain neoplasms induced by MNU.

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