

INDUCTION OF MENINGEAL TUMORS IN CHICKENS WITH AVIAN  
MYELOBLASTOSIS VIRUS

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The deliberate obtaining of experimental meningeal tumors in inexpensive and convenient small laboratory animals would provide a model of the utmost importance for research. In our view the solution of this problem could lie in the fact that viruses have affinity for particular tissues. To test this hypothesis we used mesenchymotropic avian myeloblastosis virus (AMV) and chicken meninges, which arise from mesodermal anlagen [1].

## EXPERIMENTAL METHOD

The virus, a commercial preparation of AMV (strain BAJ-A) [4], in a titer of  $10^9$ - $10^{12}$ , was supplied by the Institute of Poliomyelitis and Virus Encephalitis, Academy of Medical Sciences of the USSR. The experimental animals were chickens within 48 h after hatching: White Leghorn (Any cross) and noninbred. To obtain meningeal tumors the chickens were anesthetized with ether and 0.01 ml of inoculum was injected from a tuberculin syringe through the cranial skin and bone into the left cerebral hemisphere, beneath the meninges; in the case of tumors of the cerebellar meninges the same volume of inoculum was injected into the left half of the occiput, into the subtentorial space. After inoculation the chickens were kept 5 or 6 at a time in wire netting cages. The birds were inspected twice a day. Chickens which died or were in an agonal state were immediately autopsied. The material was placed in neutral or acid 10% formalin solution. After fixation the brain was separated from the cranial bones, taken through alcohols of increasing strength, and imbedded

TABLE 1. Induction of Cerebral and Cerebellar Meningiomas in Chickens with AMV

Expt. No.	Breed of chicks	Number of chicks	Material	Dose, ml	Mode of injection	Meningiomas		Other diseases of the CNS		
						cerebral	cerebellar	of the vascular plexuses	tumors of brains substance	proliferation of ependyma
Experiment										
1	Any cross	23	AMV	0.01	Into the brain	12	10	6		
2	Any cross	10	AMV	0.02	» »	10	8			
3	Noninbred	75	AMV	0.01	» »	25	17	16		2
		108				47	35	22		
Control										
4	Any cross (7 days old)	3	AMV	0.01	Into the brain	—	—	—	—	—
5	Any cross	11	AMV	0.1	Intravenously	—	—	3	—	2
	White Leghorn	3	AMV	0.1	» »	—	—	—	—	—
	Noninbred	7	AMV	0.1	» »	—	—	—	2*	1
	Any cross	4	AMV	0.01	Into the brain	—	—	—	3**	—
	Noninbred	6	Healthy chicken serum	0.01	» »	—	—	—	—	—
Total		142				47	35	25	5	5

Legend. \*) Undifferentiated brain tumors, \*\*) Rous sarcomas, RSV) Rous sarcoma virus.

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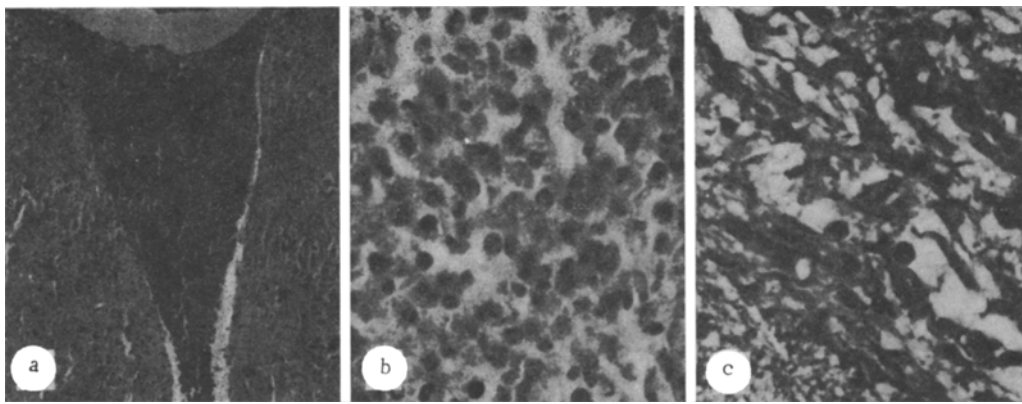


Fig. 1. Benign cerebral meningiomas: a) meningioma arising between the cerebral hemispheres. 70×; b) the same preparation. 700×; c) meningioma on lateral surface of brain. 450×; stained with hematoxylin and eosin.

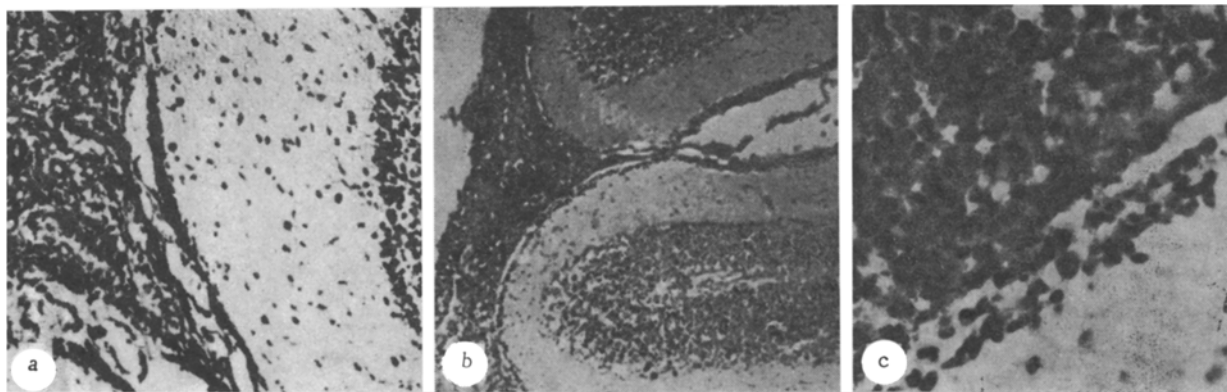


Fig. 2. Benign cerebellar meningiomas: a) meningioma growing on surface of cerebellum and wedging itself between its lobules. 90×. Stained with cresyl violet by Nissl's method; b) meningioma of cerebellum and occipital lobe of brain. 70×; c) the same preparation. 700×. Stained (b, c) with hematoxylin and eosin.

in paraffin wax. Sections 4-5  $\mu$  thick were stained with hematoxylin and eosin, by the methods of Nissl, Van Gieson, Snetsarev, Foot, and Mallory, and with orcein for elastic fibers.

#### EXPERIMENTAL RESULTS

Meningeal tumors were induced in 12 experiments on 142 chicks aged 1-2 days, including 54 White Leghorn (Any cross) and 88 noninbred (the control consisted of 18 Any cross, 3 White Leghorn, and 13 noninbred chicks). A few minutes after inoculation most of the chicks came to, fought actively, and took food and water; only a few birds remained motionless and appeared stunned for a time. Clinical signs of the disease were usually observed after 7-9 days. As a rule the affected chicks ceased to move and acquired an ungroomed appearance, and after 1-2 days drooping of one or both wings was observed. An almost characteristic pose then appeared: the wings spread out, one leg spastically extended and the other flexed, the head dipped, and the beak resting on the floor of the cage. After the first clinical manifestations appeared, death was usually fairly rapid — after 3-4 days.

Table 1 gives the yield of meningiomas and the results of the control experiments. Clearly meningiomas developed in 22 (66.7%) of the 33 Any cross chicks and in 25 (33.3%) of the 75 noninbred chicks inoculated intracerebrally with AMV. Cerebellar meningiomas also were obtained in many of the inbred and noninbred chicks inoculated intracerebrally with AMV.

Cerebellar meningiomas were found in 18 of 33 inbred chicks infected with AMV intracerebrally in doses of 0.01 and 0.02 ml. The dose of virus had a direct effect on the yield of tumors. For instance, with a dose of 0.01 ml the yield of meningiomas was 43.4%, compared

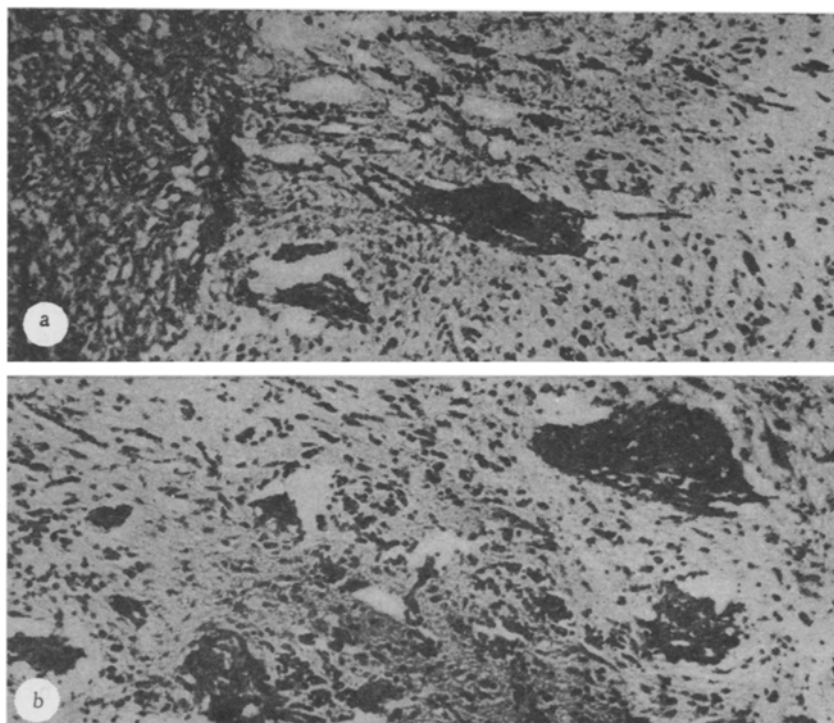


Fig. 3. Malignant cerebral meningiomas: a) invasion of and metastasis formation in brain tissue. 90×; b) multiple metastases in brain tissue. 90×. Stained with cresyl violet by Nissl's method.

TABLE 2. Microscopic Forms of Cerebral Meningiomas Induced in Chickens with AMV

Expt. No.	Breed of chicks	Number of cerebral meningiomas	Microscopic forms of cerebral meningiomas				
			meningioma-tous	fibroblastic	mixed	angioma-tous	anaplastic
1	Anya cross	12	5	3	2	1	1
2	Anya cross	10	5	1	2	1	1
3	Noninbred	25	14	8	3	—	—
Total		47	24	12	7	2	2

with 80% with a dose of 0.02 ml. In a dose of 0.01 ml the virus induced meningiomas in 17 (22.6%) of 75 noninbred chicks. Besides meningiomas, some chicks developed chorionepitheliomas, sarcomas, and undifferentiated tumors. In a group of 33 Anya cross chicks CNS tumors were found in three cases, compared with 27 cases in a group of 75 noninbred chicks.

Quite different results were obtained in the control groups. The "age control," for instance, showed that, other conditions being the same, Anya cross chicks aged 7-8 days do not develop meningiomas either after intravenous injection of the virus or after intracerebral injection of blood serum from healthy chicks. Tumors of the CNS that were not meningiomas were obtained in some control groups. For example, after intracerebral inoculation of four chicks with Rous virus, three developed sarcomas.

The morphological types of the meningiomas are given in Table 2. Although a detailed description of the cellular structure of the meningiomas will not be given, it is important to emphasize that the morphological pictures of the cerebral and cerebellar meningiomas in the same bird were analogous.

Thus AMV was found to possess the hitherto unknown property of inducing tumors of various CNS tissues. The tumors most frequently found were cerebral and cerebellar meningiomas. The pathomorphological types of meningiomas differed, but on the whole they corresponded to the WHO classification of these tumors [2, 3]. Some types included in the WHO classification were not found in our material. The absence of psammomatous meningiomas was evidently accounted for by age of the birds (they were too young) and by the rapid course of the disease. In the frequent meningotheiomatous meningiomas there was often a tendency toward the formation of concentric figures. Fibroblastic meningiomas were characterized by intensive foci of proliferation of fibrous tissue containing comparatively few cells.

Mixed meningiomas contained about equal numbers of cells of fibroblastic and meningotheiomatous forms. Meningiomas in which tumor cells arose around blood vessels, forming cuffs, were very interesting. During development of the tumor its cells invaded the vessel wall and a group of concentrically arranged cells appeared at that site. Signs of hyalinization were sometimes observed in the center of these concentric figures. It can be tentatively suggested that the meningiomas just described have a mechanism of formation similar to that of hemangiopericytic meningiomas in man. The mutual arrangement of the tumors and the brain is of great interest. In the cerebrum, more or less flat (saucer-shaped) meningiomas merely compressed the brain substance without damaging its structure (Fig. 1: a, b, c). Nodular meningiomas pressed into its substance, forming conspicuous depressions. Meningiomas of the cerebellum covered the surface of its lobules in strongly developed layers and wedged themselves between the lobules (Fig. 2: a, b, c). If a meningioma began to form between the lobules, on section it was spindle-shaped in appearance. As a rule the meningiomas simply "surrounded" the lobules of the cerebellum without penetrating into their substance.

Anaplastic meningiomas invaded the molecular layer of the cortex and spread along blood vessels quite deeply into the brain tissue (Fig. 3a, b).

In conclusion it must be emphasized that no metastases or primary tumors [6] were found in the internal organs of either the experimental or the control chickens. It is evident from the data obtained that avian myeloblastosis virus (strain BAJ-A) gives rise to tumors of the cerebral and cerebellar meninges in young birds (chickens) in a high percentage of cases [7].

This conclusion is evidence in support of the viral etiology of the origin of meningiomas, although other factors also undoubtedly exist: trauma, chemical carcinogens, radiant energy, etc. [5], which may be capable of inducing diseases such as these.

Thus avian myeloblastosis virus induces meningeal tumors in a high percentage of cases in White Leghorn (Any cross) chicks and less frequently in noninbred chicks. According to the WHO classification meningiomas induced by AMV belong to five principal types: meningotheiomatous, fibroblastic, mixed, angiomas, and anaplastic. Each type of meningioma in chicks corresponds in its basic features — shape, cell composition, relations with underlying tissues — to its human analog. Furthermore, in a small percentage of cases other CNS tumors also may be found. The avian myeloblastosis virus was shown to possess a hitherto unknown property — that of inducing other CNS tumors besides meningiomas.

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