ATAXIA IN THE NEONATALLY THYMECTOMIZED RAT:
A MORPHOLOGIC STUDY OF THE CENTRAL
NERVOUS SYSTEM†

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A syndrome in the human in which ataxia is a prominent feature has been recently associated with congenital atrophy or dysfunction of the thymus gland (1–5).

In 1941, Louis-Bar described an isolated case of progressive cerebellar ataxia associated with oculocutaneous telangiectasia (6). Boder and Sedgwick (7–13) did much to increase our knowledge on the nature of this condition that they labeled ataxia-telangiectasia. They emphasized the familial nature of this disorder, to which they added the third major component, namely recurrent severe sinopulmonary infection.

The suggestion has been offered that the latter may be linked to an altered immune response, a concept which finds support in the observation that patients with ataxia-telangiectasia bear varying degrees of deficiency in γ1-A Globulin, with normal or elevated levels of γC and γM Globulins (14, 15, 3, 16). Concurrently it has been shown that the thymus in many instances is small or absent, or deficient in lymphocytes and Hassall’s corpuscles or show changes duplicating the embryonic epithelial thymus. In individual cases, in conjunction with the thymic changes, the lymph nodes were seen to be hypoplastic (4), and lymphopenia has been recorded in many cases (5–17). Impressive similarities have been presented between ataxia-telangiectasia and one form of Swiss-type agammaglobulinemia (18). Thus, the hypothesis that the immunologic defect is due primarily to a failure of normal embryonic development of the thymus gland.

In the light of these data, it is of some interest that ataxia has been recorded in some animal species following thymectomy in the neonatal period (19). The cause of the ataxia has not been thoroughly investigated, probably because the manifestations of this disorder lack specificity and the relatively short life span of the wasted animal has not permitted any reasonable assessment of the neurological findings.

The present report deals with the morphologic study of the central nervous system (CNS) in a group of rats which developed persistent manifestations of ataxia following neonatal thymectomy.

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MATERIALS AND METHODS

The experimental group consisted of litters of rats of the W/Flu strain comprising totally thymeectomized, sham-thymeectomized, and unoperated controls. Thymeectomy was performed between the first and second day from birth. Under intraperitoneal Nembutal anesthesia (Pentobarbital sodium, Abbott, 0.75 grams per milliliter; 0.1 milliliter per 100 gram body weight) the animal was secured to a board. Using a thin-blade scalpel, skin and underlying sternum were split in the midline, from the sternum notch down to the level of the second or third rib. After widening the incision with an iris forceps the thymus was removed by mechanical suction, aided by blunt dissection of its fascial attachments in the anterior mediastinum. Bleeding was minimal and readily controlled by sterile cotton applicator sticks. After removal of the gland, the sternum was closed with a single suture and the skin by plastic adhesive dressing (Aeroplast, Aeroplast Corp., Dayton, Ohio). In all instances the removed thymus was verified for completeness under binocular, stereoscopic microscope. Of the 104 animals which were thymeectomized, 32 were lost due to infection or cannibalism. Of the remaining 72 animals, approximately one-half showed manifestations of wasting disease, and of these 9 developed ataxia. At death the CNS was examined. Approximately 2 cc of Lillie's neutral buffered formalin were injected through the fontanelles into the cranial cavity. Then, 24 hours later, with the aid of binocular stereoscopic microscope, the brain and spinal cord were removed and placed in a fixative (neutral buffered formalin) for about a week. After fixation, representative blocks of tissue were obtained from coronal sections of the cerebral hemispheres (including the basal ganglia), the brain stem and different levels of the spinal cord. The paraffin-imbedded sections were stained by the hematoxylin and eosin method, the cresyl violet method for nerve cells (Nissl); the Cajal reduced silver method for nerve fibers and the Spielmeyer method for myelin sheaths were applied to cryostat sections. The cerebellum was imbedded in toto, sectioned serially, and the consecutive sections were stained alternately by the above methods. Using the binocular stereoscopic microscope the eyes were removed and the bulbar conjunctiva was examined for telangiectasias. Possible vascular tegumental disorders were looked for in other organs and tissues (figs. 1-9).

The same procedures were applied to 9 thymeectomized “non-wasted” animals and to an equal number of sham-thymeectomized and normal controls from the same litter. Thus, a total of 36 rats were included in this study. Evaluation of the effects of thymeectomy on the humoral antibody response, and assessment of delayed hypersensitivity response to antigen administration (heterograft rejection) were attempted, but the rapid demise of the animals prevented this part of the investigation to be brought to any satisfactory conclusion.

RESULTS

Ataxia was considered to be present when the animal displayed failure of muscular coordination resulting in unsteadiness of gait. In the 9 rats which developed ataxia, the manifestations of this condition became apparent between the eighth and the twelfth day after thymeectomy, and persisted until the end of the disease, which was characterized by profound lethargy, labored respirations, and death. In every instance the ataxia was not a primary or isolated disorder, but occurred in conjunction with the distinct clinical, hematologic, and pathologic features of post-thymeectomy “wasting disease”. The survival period of the wasted-ataxic rats was considerably shorter than that of the “wasted” rats without manifestations of ataxia. They expired within 29 days from the time of thymeectomy in contrast to the “wasted”
nonataxic rats, the life span of which extended to a maximum of 40 days. None of the 104 sham-thymectomized and nonoperated controls from the same litters showed neurologic disorders.

The manifestations of "wasting" concurrent with ataxia have been previously presented (20). Briefly, they consisted of retarded growth, progressive weight loss, ruffled appearance, hunched posture, loss of hair, and peri-orbital edema. Leukopenia, reduction in circulating lymphocytes, and atrophy of Malpighian corpuscles and lymphoid tissues elsewhere were consistent additional features.

No abnormal vascular patterns, either in the bulbar conjunctiva or in the tegumental system, were noted. However, the meningeal blood vessels, both of the cerebrum and cerebellum, particularly of the latter, showed prominent vascular engorgement and dilatation. Both brain and spinal cord were otherwise not grossly remarkable, and no statistically significant differences in brain weight were noted between the ataxic-wasted thymectomized animals, the non-ataxic wasted, the sham-thymectomized, and the non-operated control animals. The cerebellum of the ataxic animals was symmetrically developed and of a size comparable to that of the other animals of the same litter. Changes were noted in the observation of the microscopic sections.
The major alterations were noted in the layer of Purkinje cells, in the inner granular and the molecular layers.

Several folia showed rarefaction or total absence of Purkinje cells in some areas and irregular alignment of the survivors in some others; not infrequently Purkinje cells were dislocated into the molecular layer. In corresponding sections, stained by the Cajal method, Purkinje cells displayed fewer arborizations than corresponding cells of the control animals, with variations in thickness both of axons and dendrites; the dendrites often exhibited terminal club-shaped expansions with short filamentous processes and twisting of the axons, as well as variations in the intensity of silver impregnation; empty basket fibers were not uncommon. Occasional cells were dwarfed in comparison to the size of adjacent, apparently normal cells, with scanty cytoplasm in proportion to the nucleus, suggesting a retardation in development. In Nissl preparations the cells were frequently vaguely outlined and there was obscuration of cell details with deviations from the normal flask shape.

The inner granular layer displayed marked variations in thickness, and thin areas irregularly alternated with areas of homogeneous thickness. In the thin areas there was concurrent rarefaction of cells with occasional variations in cell size. In conjunction with these changes, several blood channels within the folia revealed prominent telangiectasia and probably irregularities in number and distribution, which were not noted in the sections of the control animals.

No significant deviations from the normal were noted in the outer molecular
layer. Similarly, in the Spielmeyer stained sections no differences in patterns of myelination were noted either in the brain or in the spinal cord between the ataxic-thymectomized-wasted rats, the thymectomized-non-ataxic-wasted rats, and the control group of normal animals. The remainder of the brain,
Fig. 5. Prominent telangiectasia concurrent to thinning of molecular layer and absence of Purkinje cells; 28-day old thymectomized-ataxic-wasted rat. Nissl stain; 200 x.

Fig. 6. Telangiectasia and probably irregularities in number and distribution of blood channels concurrent to rarefaction of molecular cells and absence of Purkinje cells; 24-day old thymectomized-ataxic-wasted rat. Nissl stain; 200 x.
including cerebral cortex, brain stem and basal ganglia, showed also prominent telangiectasia and occasional extravasation of red blood cells. Not infrequently individual nerve cells or groups of cells, both in the cerebral cortex and basal ganglia, showed non-specific degenerative changes, and “ghost cells” were occasionally noted. Manifestations of reactive gliosis were conspicuously absent.

**COMMENT**

The cerebellum is related to the maintenance of equilibrium and to the coordination and strength of muscular movements. Upon loss of these functions, the ensuing disorder is mainly characterized by ataxia. The latter can be acute and transitory or relatively fixed, and progressive in severity. The acute transitory group is usually of toxic or infectious origin, in contrast to the relatively fixed type which may be variously related to prenatal, natal or post-natal conditions. The ataxia noted in our thymectomized wasted rats apparently belonged to the latter category. It was not possible to evaluate whether the ataxia was an isolated manifestation or just part of a more widespread neurologic deficit.

On studying the microscopic sections, the changes were found predominantly in the cerebellum, and the Purkinje cells and the molecular cell layer were seen to be primarily affected. One can assume that these changes were responsible for the ataxia.

Although arrested or retarded bodily growth is one of the major manifestations of the “wasting” that follows removal of the thymus gland in the neonatal period, we feel far less certain in placing the structural cerebellar disorder in a primary form of developmental atrophy. This hesitation arises from the well-known vulnerability of the Purkinje cells and to some extent of the granular layer of the cerebellum to systemic disturbances, primarily anoxia, which may lead to widespread destruction of cells.

The vascular component of the ataxia-telangiectasia complex in the human was not exactly duplicated by our thymectomized animals. No disorders in vascularity were noted in the bulbar conjunctiva, or in the tegumental system. However, prominent telangiectasia and probably alterations in quantity and distribution of blood channels were shown in the meningeal blood vessels. It is well possible that some relationship interceded between these vascular disorders and the changes noted in the neurons, primarily within the cerebellar folia. The linking factor might be found in stasis anoxia. This is difficult to prove; nonetheless, probably it is not accidental that vascular patterns not too dissimilar from those noted in this group of ataxic animals have been recorded by one of us in mice fetuses subjected to intrauterine anoxia (20).

In an attempt to relate all features of ataxia-telangiectasia in the human, Peterson, Kelly and Good (3) have suggested that failure of normal embryonic induction of the thymus may result in a primary defect of the mesenchyme which, in turn, may account for the telangiectasias and for the progressive neurologic alterations that they attributed to the vascular disorder.
Whereas this concept has been repeatedly challenged in the human (5, 21, 23), it might be given consideration in attempting to relate the puzzling association in our animals of neurologic disorders, abnormal vascular patterns, and suppression of thymic function.

SUMMARY

Ataxia-telangiectasia has been recently linked to an immunologic defect supposedly retsing on failure of normal embryonic development of the thymus gland.

This report deals with a morphologic study of the CNS in a group of rats of the W/Fu strain which developed manifestations of persistent ataxia following thymectomy in the neonatal period. Neuronal changes were found, primarily in the Purkinje cells and in the molecular cell layer of the cerebellum. The vascular component of the ataxia-telangiectasia complex in the human was not exactly duplicated by the thymectomized animals; however, prominent telangiectasia and probably alterations in quantity and distribution of the meningeal blood vessels were noted. The puzzling association of neurologic disorders, abnormal vascular patterns, and suppression of thymic function is discussed.

REFERENCES


