sites" and the speed with which it accumulates at such sites, regardless of whether it forms *in situ* or arrives preformed, may be the principal determinants of shock. The concept that the primary action of the soluble antigen-antibody complex is at extravascular sites and involves complement is compatible with the proposition that anaphylaxis in the mouse results from the rapid release of shock "mediators" from tissue mast cells (*16*).

In summary, it should be emphasized that much additional evidence will be necessary to establish the site at which primary cell injury is produced by the action of antigen-antibody complexes and the ensuing events which lead to anaphylaxis.

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29 November 1957

Production of Injury to Feline Central Nervous System with Nucleic Acid Antimetabolite

Biochemical and autoradiographic investigations in this laboratory have shown that neurons and glia within the mature mammalian neuraxis actively incorporate C^{14}-labeled adenine and orotic acid into pentose nucleic acid (*1*). It appears likely that this is related to the turnover of protein which occurs in nerve cells (*1, 2*) and glia (*1*).

In order to test this hypothesis and to investigate the function of pentose nucleic acid in the central nervous system, we have attempted to interfere with pentose nucleic acid turnover by the use of nucleic acid antimetabolites. These substances, administered orally and parenterally, have been employed extensively in animal experimentation and in the treatment of neoplasms and blood dyscrasias in man. Acute and transient disturbances such as ataxia, weakness, and convulsions have occurred when massive doses are given to animals (*3*), but no delayed and lasting neurological symptoms have been reported. It is possible that the blood-brain barrier protects the central nervous system from these antimetabolites. In the present study this obstacle was circumvented by the expedient of injecting the antimetabolites into the subarachnoid space. In the course of this investigation, a pyrimidine antimetabolite was found to produce a consistent and striking neurological syndrome in cats. This, together with preliminary neuropathological and histometabolic observations, constitutes the subject of this report.

A fluorinated pyrimidine, 5-fluoroorotic acid (*4*), was used because of the previously demonstrated incorporation of orotic acid into pentose nucleic acid in the feline neuraxis (*1*). The biological activity and tumor-inhibitory properties of this substance have been described by Heidelberger *et al.* (*5*). 5-Fluoroorotic acid or its sodium salt (20 to 30 mg/ml in water; because of its poor solubility, it is used as a supersaturated solution which is heated and cooled to body temperature at time of injection) in a single dose of 3 to 10 mg is administered by intracisternal injection to cats anesthetized with pentobarbital sodium or ether. In some animals a second injection is given about 1 week after the first, to make a total dose of 5 to 15 mg.

Following an initial symptom-free period of 5 to 10 days, the cats undergo a change in behavior. They become somewhat sluggish and inactive. Within several days an unsteadiness in walking becomes apparent. This rapidly progresses and becomes a severe ataxia involving the trunk and extremities. There is no gross paralysis, but all movements are marred by typical cerebellar oscillations. Animals walk with a lurching, reeling gait. In advanced stages of this condition they are so helpless that they are unable to eat or move about. Polypnea is frequently present, and nystagmus is occasionally seen, particularly on rapid head movement. At times, twitchings of the face and vibrissae appear which resemble the fasciculations of motor neuron disease; these are abolished by pentobarbital anesthesia. Tonic and clonic convulsive movements have been noted preterminally in several immature cats.

No attempt has been made, thus far, to nurse animals beyond the tenth day of overt illness; two animals died of this illness, probably because of respiratory or bulbar involvement, but the remainder were killed. No evidence of hematologic or gastrointestinal involvement was noted. This contrasts with the reported results of oral and parenteral administration of nucleic acid antimetabolites, where these systems are involved (*3*). In control studies, orotic acid had no deleterious effect when given in doses similar in amount. Neurological symptoms were not observed when the drug was given in large doses by intraperitoneal injection.

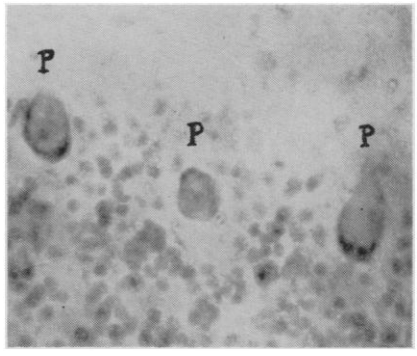

Fig. 1. Cerebellar cortex from a cat with severe cerebellar ataxia produced by the intracisternal injection of 5-fluoroorotic acid 17 days before sacrifice. Note the perinuclear depletion of basophilic substance, only a peripheral rim persisting in the cytoplasm of the Purkinje neurons (*P*). (Gallocyanin chromalum stain) (about ×360).

Histological examination of the brain and spinal cord of these animals has not been completed. However, a striking lesion has been found in the cerebellum in all of six brains examined to date. In routine Nissl– and hematoxylin-eosin–stained sections, most of the Purkinje neurons of the cerebellar cortex revealed advanced central chromatolysis (Fig. 1). Thus the severe cerebellar deficit exhibited by these animals can be ascribed to Purkinje cell damage. Occasional neurons of the brain stem and spinal cord showed similar though less marked alterations. This may account for the other neurological symptoms noted, such as polypnea and convulsions.

Studies have been completed on two fluoroorotic acid treated cats which were given L-methionine-S^{35} in doses of 100 μc (specific activity 10 to 14 μc/mg) 4 hours before sacrifice. Autoradiographs prepared from formalin-fixed, acid- and lipid-extracted tissue sections of brain and spinal cord (*1*) showed marked reduction in the radioactivity of neurons and glia by comparison with untreated animals. However, the intracisternal route of injection may not give quantitatively reproducible results, and only tentative conclusions can be drawn from this evidence alone. The spinal ganglion neurons in these preparations, on the other hand, exhibited a relatively nor-

mal degree of radioactivity; in untreated animals under these conditions, spinal ganglion neurons give a less intense autoradiograph than nerve cells within the neuraxis (*1*). These observations provide evidence that, in cats treated with fluoroorotic acid, the ability to incorporate labeled methionine into the protein of nerve cells and glia within the neuraxis is impaired but that the ability to incorporate it into the protein of spinal ganglion neurons is not.

Several considerations make it seem likely that the effects of intracisternal injection of 5-fluoroorotic acid in cats result from an interference with pentose nucleic acid metabolism. The chromatolysis of Purkinje neurons itself represents a quantitative reduction of cytoplasmic pentose nucleic acid (*6*). The course of this syndrome—that is, the symptom-free latent period, gradual onset, and progressive worsening—resembles the toxic effect of nucleic acid antimetabolites on bone marrow, liver, intestinal mucosa, and tumors (*3*). The apparent diminution in incorporation of labeled methionine into nerve and glial cell protein furnishes further support for this hypothesis, since pentose nucleic acid plays an important role in protein synthesis (*7*). The significance of this evidence is enhanced by the finding that spinal ganglion cells show little reduction in incorporation of labeled methionine; 5-fluoroorotic acid would be expected to have much less effect on these cells because they incorporate orotic acid into pentose nucleic acid at a relatively slow rate (*1*). Possible mechanisms of action of 5-fluoroorotic acid include: (i) competitive or noncompetitive blocking of the incorporation of normal precursors into pentose nucleic acid, leading to depletion of the acid; and (ii) the incorporation of the fluorinated pyrimidine analog or its derivatives into pentose nucleic acid, rendering it metabolically ineffective or harmful. The proximity of the cerebellum to the site of injection and its active turnover of pentose nucleic acid probably account for the early and severe derangement of this portion of the neuraxis. The production of neurological deficit, neuronal lesions, and impairment of protein metabolism in nerve and glial cells by a nucleic acid antimetabolite indicates the possibility that some "degenerative" and other disease processes which occur within the central nervous system may be caused by biochemical or other disturbances in nucleic acid metabolism (*8*).

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Relation of Structure to Adsorption of Substances during Crystallization of L(−) Tyrosine

The incorporation of impurities into growing crystals has attracted considerable attention for a long time. Whereas the prevailing emphasis has been directed toward modification of crystal growth or habit, virtually nothing has been said of the relationship between chemical structure and incorporation.

With L(−)tyrosine as the parent substance, and chemically related substances as the impurities, the incorporation by the growing crystals was studied by the use of the spectrophotometric method of Goodwin and Morton (*1*). The data, illustrated in Fig. 1, demonstrate that the incorporation of the various substances used is specific. No incorporation was observed with hydrocinnamic acid (not shown) up to 0.016*M*, where solubility difficulties are encountered. In the case of L(−)alanine (not shown), a concentration of 0.047*M* was required before incorporation could be detected [0.041*M* L(−)alanine per mole of tyrosine].

The adsorption process is clearly evident in the cases of L(−)histidine, D(+)-phenylalanine, and L(−)phenylalanine. The amount of impurity incorporated is seen to be related not only to the structural similarity of the mother substance and the impurity but also to the spatial relationships of the two substances, as evidenced by the stereoisomeric specificity of the phenylalanine isomers.

For L(−)phenylalanine, the adsorption empirically resembles that of Henry's law or a simple Langmuir isotherm, which is indistinguishable from it at small adsorptions. It is possible to conceive of the growing crystal, because of its ability to present a changing surface to its surrounding medium, as obeying the Langmuir isotherm for a given surface and Henry's law for the sum total of surfaces generated, resulting in what is known as a solid solution. In this instance the heat of adsorption and the adsorption capacity of each new surface would have to remain constant. When this does not occur, deviations from linearity would be expected. This, perhaps, is part of the exacting requirements necessary for specificity of a stereoisomeric adsorption.

Additional information concerning this specificity is obtained from the examination of the molecular models of the interacting substances. These are shown in Fig. 2. Models with the same configuration are depicted as L. The stereoisomer of this configuration is arbitrarily designated D. If we assume that the complementary charged groups of the two interacting substances are active in the adsorption process, the charged amino group of one is placed in the position of closest approach to the carboxylate ion of the other, the rings of both molecules being in the same plane. In the case of L(−)tyrosine and D(+)phenylalanine, the two carboxylate ions are capable of very close approach, and hence one would anticipate an inhibitory effect from the resulting repulsion. This is actually reflected in the decreased adsorption at higher concentrations (Fig. 1). In the case of L(−)phenylalanine and L(−)tyrosine, this proximity of the carboxylate ions does not exist. In fact, it is possible that there is a hydrogen bond between the two carboxylate ions, thus enhancing the adsorption.

These experiments indicate that no one bond type is responsible for the adsorption by tyrosine. The ionic bonds, which are the most important in the crystal, as evidenced by the high melting points of dipolar ions generally, must be supplemented by other forces. With the exception of hydrocinnamic acid, all the substances studied may be looked upon as derivations of alanine. Hence, if the

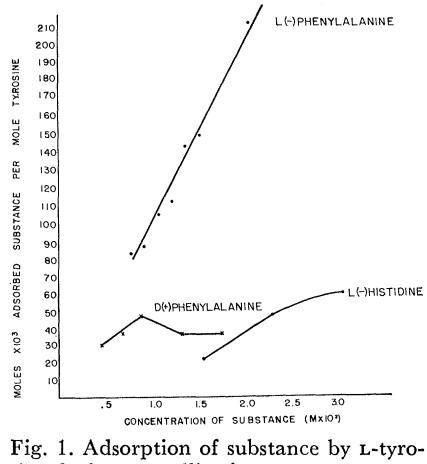

Fig. 1. Adsorption of substance by L-tyrosine during crystallization.