Reports

Production of Anaphylaxis in the White Mouse with Soluble Antigen-Antibody Complexes

Definitive information on anaphylaxis is still lacking with respect to the tissue sites at which the causative antigen and antibody interact and the mechanism by which antigen-antibody interaction leads to shock.

Germuth and McKinnon (*1*) have recently made the important observation that soluble complexes of antigen and precipitating antibody produce immediate anaphylactic shock in the guinea pig. This anaphylactigenic action of soluble antigen-antibody complexes is compatible with the earlier observation that anaphylaxis can be produced with "univalent" antibody which forms only soluble complexes with antigen (*2*, p. 97). The findings of Burdon (*3*) that severe passive anaphylaxis in mice could only be produced by intravenous challenge with antigen when antibody was still abundant in the blood or by the intravenous injection of "suitably proportioned" antigen-antibody mixtures may have depended on the anaphylactigenic action of soluble antigen-antibody complexes. Since the use of soluble antigen-antibody complexes presents a new approach for investigating the mechanism of anaphylaxis, it appeared desirable to study the action of these complexes in the mouse, in which the course of anaphylaxis differs from that observed in the guinea pig (*4*).

The mice used were of mixed sexes of a white Swiss strain. They were from 6 to 9 weeks old and ranged from 22 to 27 g in weight. The antigen employed was crystalline bovine serum albumin (BSA) dissolved in physiological saline (*5*). The heat-inactivated rabbit antiserum that was used contained 2.32 mg of antibody N per milliliter (*6*). It was produced by the method of Freund (*7*) from heat-killed bacillus Calmette-Guérin.

The first method used to prepare soluble antigen-antibody complex was to add antiserum to excess antigen. The quantity of antigen used was eight times the amount determined to be present in a precipitate formed in the zone of equivalence. The antigen-antibody mixtures were incubated at 37°C for 20 minutes and cleared by centrifugation. The second method of preparing soluble antigen-antibody complex was that of using excess antigen to dissolve a precipitate obtained in the zone of equivalence. The precipitate was washed four times in physiological saline at 4°C, and a concentrated solution of antigen was added, containing a total amount of antigen equal to ten times that present in the precipitate. This mixture was agitated and incubated for 40 minutes at 45°C and centrifuged to remove the small amount of undissolved precipitate. All preparations were injected into the tail veins of mice in a volume of 0.4 ml. In the first experiment, a dose of soluble complex prepared by the first method and containing 0.6 mg of antibody N, produced anaphylactic death in 15 of 16 mice. A dose of soluble complex prepared from a precipitate by the second method and containing 0.48 mg of antibody N produced anaphylactic death in 10 of 16 mice. Control preparations were: BSA in normal serum, anti-BSA serum, bovine gamma globulin (*5*) in anti-BSA rabbit serum, BSA alone, and supernatant fluids from mixtures of antigen and antibody in the zone of equivalence. The number of animals in the groups given the control preparations varied from 6 to 13.

Whereas animals of both test groups reacted uniformly, with typical severe anaphylaxis (*8*) within 2 to 5 minutes and deaths occurred in from 15 to 35 minutes, the animals that received control preparations were not affected. Although the lower mortality in the mice which received the complex prepared from washed BSA–anti-BSA precipitate was presumably due to the lower dose of complex given these animals, an alternative possibility is that the antigen-antibody aggregates derived from washed precipitates were larger (*9*) and less anaphylactigenic.

In the next experiment mice were injected intraperitoneally with 0.1 ml of *Hemophilus pertussis* vaccine (*10*) (48.8×10^9/ml in 1:10,000 merthiolate) 5 days before challenge with antigen-antibody complex. Whereas the injection of a dose of soluble complex prepared by the first method and containing 0.12 mg of antibody N, caused anaphylactic death in 18 of 18 pertussis-vaccinated animals, only 5 of 18 nonvaccinated animals died. This marked increase in the susceptibility of the pertussis-vaccinated mice to shock with the complex is similar to observations which have been made in pertussis-vaccinated mice sensitized actively (*11*) and passively (*12*) and shocked with antigen in the usual manner.

Because it is unlikely that the antigen-antibody complexes used underwent significant dissociation in vivo (*1, 13*), anaphylaxis was presumably caused by soluble antigen-antibody complexes acting either within the circulating blood or in surrounding tissues into which they may have diffused.

Since it is probable that soluble antigen-antibody complexes formed by either nonprecipitating or precipitating antibody in the region of antigen excess consist principally of aggregates of two or three molecules (*14*), the size of the aggregates may not be sufficient to prevent, or perhaps even markedly retard, their passage to extravascular sites (*15*). The observation that anaphylaxis produced with soluble antigen-antibody complexes was typical in all respects suggests that the site of antigen-antibody action and ensuing events leading to anaphylaxis are the same regardless of whether shock is induced in the usual manner by injection of antigen or by the injection of soluble preformed antigen-antibody complexes.

The results reported here do not support or refute the suggestion of Benacerraf and Kabat (*2*, p. 517) that the "time-consuming reaction" responsible for the incubation period necessary for maximum sensitization to passive anaphylaxis in the guinea pig represents the time necessary for the fixation of antibody to cells, for there is no evidence to preclude the possibility that the antigen-antibody complex itself must "fix" to cells in order to produce shock. Neither do the present results justify any change in the common opinion that the speed and severity of shock are determined by the rate at which antigen reaches "fixed antibody"; for the rate at which preformed soluble antigen-antibody complex reaches the sites where antigen-antibody injury is produced may likewise determine the speed and severity of the resulting shock. Indeed, the amount of the antigen-antibody complex at "injury

All technical papers are published in this section. Manuscripts should be typed double-spaced and be submitted in duplicate. In length, they should be limited to the equivalent of 1200 words; this includes the space occupied by illustrative or tabular material, references and notes, and the author(s)' name(s) and affiliation(s). Illustrative material should be limited to one table *or* one figure. All explanatory notes, including acknowledgments and authorization for publication, and literature references are to be numbered consecutively, keyed into the text proper, and placed at the end of the article under the heading "References and Notes." For fuller details see "Suggestions to Contributors" in *Science* 125, 16 (4 Jan. 1957).

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.

SEIKICHI TOKUDA
RUSSELL S. WEISER
University of Washington School of Medicine, Seattle

References and Notes

1. F. G. Germuth and G. E. McKinnon, *Bull. Johns Hopkins Hosp.* 101, 13 (1957).
2. E. A. Kabat and B. Benacerraf, *J. Immunol.* 62 (1949).
3. K. L. Burdon, *Federation Proc.* 5, 245 (1946).
4. This investigation was supported in part by the Washington State Heart Association.
5. The bovine serum albumin and the bovine gamma globulin fraction II used in this study were secured from the Armour Laboratories, Chicago, Ill.
6. Antibody N was determined by the Folin-Ciocalteu method.
7. J. K. Freund *et al., J. Immunol.* 60, 383 (1948).
8. R. S. Weiser, O. J. Golub, D. M. Hamre, *J. Infectious Diseases* 68, 97 (1941).
9. J. L. Oncley, E. D. Gitlin, F. R. N. Gurd, *J. Phys. Chem.* 56, 85 (1952).
10. The pertussis vaccine used in this study was generously supplied by the Merck Institute for Therapeutic Research, Rahway, N.J.
11. S. Malkiel and B. J. Hargis, *J. Allergy* 23, 352 (1952).
12. L. Kind, *J. Immunol.* 70, 411 (1953).
13. W. J. Kleinschmidt and P. D. Boyer, *ibid.* 69, 257 (1952).
14. W. C. Boyd *Fundamentals of Immunology* (Interscience, New York, ed. 3, 1956), p. 63.
15. A. H. Coons, *Intern. Rev. Cytol.* 5, 1 (1956); H. H. Shirley *et al., Am. J. Physiol.* 190, 189 (1957); J. M. Hampton, *Acta Anat.*, in press; H. S. Bennett, *J. Biophys. Biochem. Cytol., Suppl. 2* (1956), p. 103; G. E. Palade, *J. Appl. Phys.* 24, 424 (1953).
16. R. S. Weiser, *J. Allergy* 28, 475 (1957).

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.

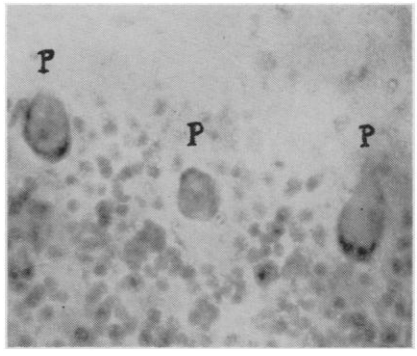

Fig. 1. Cerebellar cortex from a cat with severe cerebellar ataxia produced by the intracisternal injection of 5-fluoroorotic acid 17 days before sacrifice. Note the perinuclear depletion of basophilic substance, only a peripheral rim persisting in the cytoplasm of the Purkinje neurons (*P*). (Gallocyanin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