SPECIAL ARTICLES

VENEZUELAN EQUINE ENCEPHALOMYELITIS

As is well known, severe epidemics of equine encephalomyelitis have occurred this year in Canada and the United States. A similar disease has now broken out in northern South America. Through the courtesy of the Ministry of Agriculture of the Venezuelan government and of Dr. Gunnar Tryde, of Caracas, we have received portions of brains of animals dying of this disease.

Virus has been recovered from these brains and preliminary observations have been made on the disease it produces in guinea pigs, mice and chicken embryos. The symptoms resemble those of Eastern encephalomyelitis. Nevertheless, it is clear that this virus is significantly different from either of the known American strains. Guinea pigs receiving it intracerebrally show first signs of paralysis after about 48 hours; they died in from three to five days. In young mice the disease follows a similar course. Guinea pigs can be infected by subcutaneous inoculation. Virus dropped onto the chorio-allantoic membrane of a developing chick embryo rapidly infects the entire embryo, which dies within a day. Brains of guinea pigs moribund with the Venezuela disease are especially rich in virus. Four that we have titrated have all been infectious for mice in a dilution of 10^{-7}. This indicates a virus concentration 10 to 100 times greater than that usually found in Eastern diseased brains. Several embryo-cultured virus preparations have had dilution end-points of 10^{-8}. They thus have contained about the same amount of virus as Western strain diseased embryos; Eastern strain embryos are ordinarily more infectious.

It has been shown[1] that formalinized virus vaccines will give solid immunity against corresponding forms of encephalomyelitis. We have made similar vaccines with Venezuela virus. All guinea pigs thus far vaccinated with them have successfully withstood the intracerebral injection of massive doses of Venezuelan virus.

Cross-protection tests have been made involving Venezuelan disease on the one hand and Western and Eastern encephalomyelitis on the other. Western strain vaccinated guinea pigs injected with Venezuelan virus have all succumbed with no evidence of protection. Eastern immune pigs diseased with Venezuelan virus have also all died, though in many instances a day or so later than unvaccinated controls. In a third experiment two groups of animals protected with the Venezuelan vaccine were given 10 to 100 m.l.d. of Eastern strain virus. They all died of encephalomyelitis, but those injected with the smaller dose outlived their controls.

These results demonstrate that our Venezuelan strain of encephalomyelitis is immunologically different from the familiar Eastern and Western forms of this disease. Though they are as yet insufficient to settle the question, they suggest that a remote relationship may exist between the Eastern and Venezuelan strains. No comparisons have been possible with the viruses of Borna's disease or of Russian encephalomyelitis. Work with the Venezuelan disease is being continued.

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[1] M. S. Shahan and L. T. Giltner, *Jour. Am. Vet. Med. Assoc.*, 84: 928, 1934; P. K. Olitsky and H. R. Cox, *Jour. Exp. Med.*, 63: 745, 1936; J. W. Beard, H. Finkelstein, W. C. Sealy and R. W. G. Wyckoff, SCIENCE, 87: 89 and 490, 1938; C. E. Beck and R. W. G. Wyckoff, SCIENCE, 88: 264, 1938; A. Eichhorn and R. W. G. Wyckoff, *Jour. Am. Vet. Med. Assoc.*, 46: 285, 1938.

THE PROTECTIVE EFFECT OF SULFANILAMIDE IN MICE AGAINST GONOCOCCAL "TOXIN"

SINCE sulfanilamide was first introduced for the treatment of gonococcal infections, we have been interested in the possibility that the favorable action of the drug might be concerned with the "toxin" of the gonococcus. Although the bactericidal effect of sulfanilamide *in vitro* has previously been demonstrated,[1,2] we have recently obtained an *in vitro* inactivation of gonococcal "toxin" with the compound.[3] Clinical data indicate that factors other than a direct effect of the drug on the gonococcus may contribute to its therapeutic efficacy. Thus, it is well known that certain patients recover with a minimal concentration of the drug in the tissues, while others fail to be cured when a high concentration is present. On the other hand, it has been frequently observed that the gonococcus is still present in infected areas after the symptoms have subsided subsequent to treatment with sulfanilamide. These facts point to an interaction of sulfanilamide with a gonococcal "toxin." Although some doubt has prevailed as to the existence of a substance which can rightly be termed a "toxin," our studies substantiate the findings of earlier investigators,[4,5] whose work indicates that a "toxin" is formed by the gonococcus.

By employing a technique similar to that described by Clark, Ferry and Steele[4] for the production of gonococcal "toxin," whole cultures, filtrates and supernates were prepared from several strains of the

[1] H. F. Wengatz, R. A. Boak and C. M. Carpenter, *Jour. of Bact.*, 35: 36, 1938.

[2] A. Cohn, *Am. Jour. Syph., Gonorr. and Ven. Diseases*, 22: 1, 1938.

[3] C. M. Carpenter, G. M. Barbour and P. L. Hawley, *Jour. of Bact.*, 36: 280, 1938.

[4] J. DeChristmas, *Ann. de l'Inst., Pasteur*, II: 609, 1897.

[5] L. T. Clark, N. S. Ferry and M. H. Steele, *Jour. Immunology*, 21: 233, 1931.

gonococcus which were fatal for mice in large doses. Because of the instability of these materials, the preparations were lyophilized by means of a Flosdorf-Mudd apparatus.[6] This procedure not only stabilized, but also concentrated the preparations, which were complex mixtures of metabolic products and autolysed cells. A regenerated lyophilized ascitic fluid broth culture of the gonococcus, containing no viable organisms, was employed in our studies. It is this type of preparation to which we give the name "toxin."

Although ascitic fluid broth cultures of several of our strains of the gonococcus killed mice, the results reported herewith concern only one particular strain, the "Le D." This strain was isolated during March, 1937, from the knee-joint of a patient with gonococcal arthritis.

After determining that the intraperitoneal injection of 0.2 cc of the regenerated lyophilized "toxin" killed mice (20 to 30 gm) consistently, a study was made of the relative toxicities and therapeutic values of various preparations of sulfanilamide. A 2 per cent. solution of the compound in 0.85 per cent. NaCl solution was found to be most satisfactory for our purposes. The sulfanilamide was administered intraperitoneally in two doses, one of 10 mg immediately after the injection of the "toxin" and a second of 20 mg 5 hours later.

After inoculation, the control mice usually died within 18 hours; a few lived longer than 24 hours. The coat became rough; the mice were listless and remained in a crouched position until death. Generally, diarrhoeae developed shortly after inoculation and the temperature became subnormal. The eyelids were often stuck together with a mucopurulent exudate.

The mice treated with sulfanilamide became cyanotic, ataxic or spastic and markedly opisthotonic. The reactions from the drug subsided in less than an hour, after which time the mice presented, to a lesser degree, some of the symptoms observed in the controls. When the drug became effective, the treated mice improved gradually, the majority recovering within 48 hours.

TABLE 1
THE THERAPEUTIC EFFECT OF SULFANILAMIDE IN MICE INOCULATED WITH GONOCOCCAL "TOXIN"

Group	No. of mice injected	Treated No.	Treated Died	Untreated No.	Untreated Died
1 ..	15	10	0	5	5
2 ..	15	10	0	5	5
3 ..	13	9	0	4	4
4 ..	16	10	0	6	6
5 ..	8	5	0	0	3
6 ..	60	50	4	10	10
Total .	127	94	4(4.3 per cent.)	33	33(100 per cent.)

Summary		Per cent. protected	Standard error
	Mice with sulfanilamide .	95.7	±2.1
	Mice without sulfanilamide	0	

Results obtained with intraperitoneal doses of 10 and 20 mg of sulfanilamide administered immediately and 5 hours, respectively, after the intraperitoneal injection of 0.2 cc of lyophilized and regenerated gonococcal "toxin" ("Le D" strain).

[6] E. W. Flosdorf and S. Mudd, *Jour. Immunology*, 29: 389, 1935.

The results obtained by averaging 6 tests are given in Table 1. Six groups of mice were used in this particular study, the number of mice per group varying from 8 to 60. The total number was 127, of which 33 were controls. All the controls died within 24 hours. Only 4, or 4.3 per cent., of 94 treated mice failed to be protected. Of these, 2 lived for 4 days after treatment.

In summary, gonococcal "toxin" (a term used to describe the regenerated lyophilized ascitic fluid broth culture of the gonococcus, containing no viable cells, used in the present studies), which killed mice following its intraperitoneal injection, was produced from several strains of *Neisseria gonorrhoeae*. In the present report the results with one strain only, the "Le D," are described.

Mice injected with lethal amounts of the gonococcal "toxin" were protected from death by the administration of adequate therapeutic doses of sulfanilamide.

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THE FEASIBILITY OF PRESERVING NEOPLASTIC CELLS IN THE FROZEN STATE[1]

STUDIES on the resistance of single-celled or small multicellular animals and plants to extreme cold are reviewed by Heilbrunn,[2] who points out that survival after freezing may be due to factors which inhibit ice crystal formation. Ice crystals injure the cell both mechanically and by withdrawing water from the protoplasm. It is generally believed that slow freezing is associated with the formation of large crystals and is more injurious than rapid freezing.

Experiments on the resistance of the more labile neoplastic cells of mammals to freezing have yielded different results in the hands of different investigators. Carcinoma cells have been kept alive by Ehrlich[3] for two years at −8° C. This temperature does not completely solidify the tissue. Ehrlich has also found that the growth of carcinoma cells is retained after exposure to −30° C. for 48 hours. Michaelis[4] has successfully transplanted Jensen's tumor after it had been exposed to liquid air for a half hour. His obser-

[1] These investigations have been supported by the International Cancer Research Foundation, the Lady Tata Memorial Trust and a Fund for the Study of Leukemia.

[2] L. V. Heilbrunn, "An Outline of General Physiology," Philadelphia and London, W. B. Saunders Company, 1937.

[3] P. Ehrlich, *Zeitschr. f. Krebsf.*, 5: 65, 1907.

[4] L. Michaelis, *Med. Klin.*, 5: 204, 1905.