

being muscular. This and the greater chemical stability of the alkaloid suggest why its actions are better sustained than those of epinephrine.

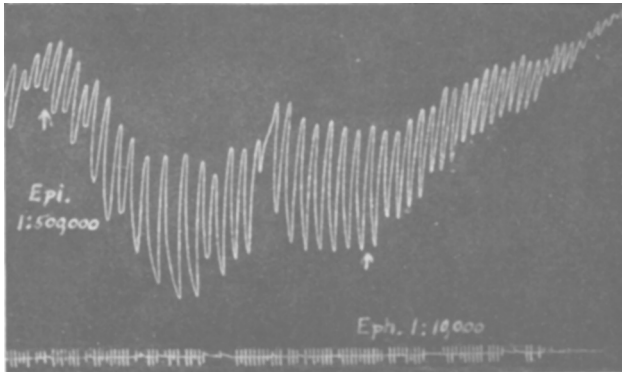


FIG. 3.

Strip of pregnant guinea pig uterus showing inhibitory response to epinephrine and augmentor response to ephedrine.

During the past two years different extracts and extractives (corresponding to alkaloid) of several specimens of *Ephedra nevadensis* obtained from Utah have been examined in this laboratory with negative results as to the presence of alkaloids and demonstrable physiological effects, except those which could be explained as due to the presence of tannin. Controls with extracts from Chinese ephedra and with ephedrine gave positive results.

¹ De Eds, F., *Proc. Soc. Exp. Biol. and Med.*, 1927, xxiv, 551.

² Kreitmair, H., *Arch. Exp. Path. Pharm.*, 1927, exx, 189.

* The ephedrine used in this work came from two sources; a sample kindly supplied by Dr. Chen, and different samples marketed by the Abbott Laboratories.

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Studies in Tuberculosis. XI. Neutralization of Filterable Tubercle Toxin (T. F.) with Sheep Antiserum (Antitoxin).

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In continuation of the experiments reported previously,^{1, 2, 3} Berkfield filtrates from broth cultures of tubercle bacilli were used in the

systematic immunization of a sheep. These toxin filtrates, hereafter designated as T. F., were obtained by growing a virulent strain of human tubercle bacilli in glycerinated "hormone broth" for 18 to 21 days and filtering the culture through a Berkfeld "N" candle. The sheep was injected subcutaneously at weekly intervals with increasing doses of freshly prepared filtrate. After four months, neutralizing properties of the serum were studied by the intracutaneous and intraperitoneal method in tuberculous and normal guinea pigs, and by skin tests in tuberculous and non-tuberculous human subjects.

Having found previously that an intracutaneous injection of unheated T. F. in the experimental tuberculous animal caused a definite skin reaction, the effect of an incubated mixture of immune sheep serum and T. F. was now studied.

Intracutaneous Method. Series of four guinea pigs each were infected with human tubercle bacilli, respectively 3 and 9 weeks and 3 and 5 months before these tests were made. Intracutaneous injections were given of a mixture of equal parts of serum and T. F., T. F. in a dilution of 1:2, immune serum alone, a mixture of normal sheep serum and T. F., and a mixture of immune rabbit serum and T. F. The dose, 0.05 cc., was injected after incubating all of these materials for one hour at 37.5° C. on a water bath. The results showed consistently that immune sheep serum (or immune rabbit serum) prevented the occurrence of positive skin tests, whereas normal sheep serum or normal rabbit serum failed to do so. Positive reactions were characterized by an area of redness and deep induration varying in extent from 0.5 x 0.5 to 1.5 x 1.5 cm. and lasting several days.

Intracutaneous Method. Series of four guinea pigs each were property of antiserum from the sheep was obtained by injecting intraperitoneally mixtures of immune serum and T. F. incubated for 1 hour at 37.5° C. Tuberculous guinea pigs reacted uniformly to an intraperitoneal injection of from 1 to 2 cc. of T. F. with the exhibition of toxic symptoms that came on rapidly following a dose of the filtrate. Dyspnea, spasmodic contraction of the diaphragm simulating hiccough, marked weakness of the extremities, and profound prostration, lasting several hours, occurred regularly in all of the animals so tested. When from two to three-fold doses of T. F. were combined with immune sheep serum the typical reactions were prevented. Normal serum failed to accomplish this result and in normal guinea pigs the T. F. alone or in combination with immune or normal sheep serum produced no symptoms of intoxication.

Skin Tests, Adults. There were 180 patients with advanced tuberculosis, of whom 84 showed clinical improvement and the re-

remainder did not. Intracutaneous tests were made with T. F. diluted 1:20 in physiological salt solution and incubated mixtures of equal parts of T. F. (diluted 1:10) and immune sheep serum. One-tenth cubic centimeter of the test material was injected into the skin.

Reactions were uniformly positive with T. F. in cases of advanced tuberculosis showing definite clinical improvement and were negative in patients who were failing and had a bad prognosis. In early tuberculosis more marked reactions were noted, and in apparently normal adults who were clinically non-tuberculous but had the usual amount of spontaneously healed tuberculous foci, skin tests were uniformly positive and intense. Immune serum combined with T. F. prevented the occurrence of skin reactions that were produced by one-half the amount of T. F. alone.

Children. This group included 60 patients, of whom 40 were definite contacts to tuberculosis and the remainder not exposed. Positive skin tests with 0.1 cc. of a 1:20 dilution of T. F. were observed in the group of contacts only. These children gave substantial evidence of tuberculosis in healed, arrested or active stages, and the severity of the skin reaction was correlated quite uniformly with the type of infection present. Tests with the ether-soluble tuberculin fraction in these children were negative. An additional series of

TABLE I. *Summary of Results.*

Group of Patients	Number	Skin Reactions		
		T. F. (dil. 1:20)	T. F. (dil. 1:5) plus equal volume of immune sheep serum	Ether soluble tuberculin fraction (dil. 1:50)
Tuberculosis (advanced)	180			
With clinical improvement	84	Positive	Negative*	
Failing, with bad prognosis	96	Negative	Negative*	
Clinically non-tuberculous adults with spontaneously healed foci	60	Positive	Negative	
"Contact" children	378			Negative
	40	Positive	Negative	Negative
"Non-contact" children	20	Negative	Negative	Negative

*16 patients tested; 13 with improvement—T. F. positive; 3 failing, with bad prognosis—T. F. negative.

378 children, tested with tuberculin fractions, gave uniformly negative skin reactions with the ether-soluble derivative. From these

results it may be deduced that the T. F. is not related to any possible bacillary substances most likely to give skin reactions. Of such elements the fatty derivatives, soluble in ether or acetone, are the ones to be controlled.

The skin tests made on adults and children and experimental animals suggest, furthermore, that T. F. offers a method for detecting existing tuberculous infection that is clinically significant, particularly in children. In this connection, ordinary tuberculin tests have not proved valuable.

The toxin filtrate of tubercle bacilli measures antitoxin content or susceptibility to tuberculous infection. A negative intracutaneous test with T. F. indicates either the absence of tuberculous foci and hence no resistance, or an advanced infection with profound toxemia and the amount of antibody entirely absent or too small for detection. In advanced tuberculosis, the reactions appeared to be correlated with the clinical condition of the patient and the prognosis in each case.

From these results and the studies previously noted, it may be concluded that the requirements for the demonstration of toxin by tubercle bacilli have been fulfilled.

Further studies are now in progress on methods of standardizing immune serum against toxin filtrates of different potency. Active immunization experiments have been begun with T. F. in animals and in children.

This is a preliminary report.

¹ Eberson, F., *PROC. SOC. EXP. BIOL. AND MED.*, 1926, xxiv, 79.

² Eberson, F., *ibid.*, 1927, xxiv, 329.

³ Eberson, F., *ibid.*, 1927, xxiv, 331.