Hemagglutination Reaction by the Phosphatide of the Tubercle Bacillus

We have found that, as shown by the hemagglutination reaction in tuberculosis, the phosphatide of the tubercle bacillus possesses an erythrocyte-sensitizing capacity as high as or higher than that of the tuberculin polysaccharide. We have also found that the antigen-antibody system participating in the phosphatide hemagglutination is completely independent of that participating in the polysaccharide hemagglutination.

Of the three phosphatide samples used, the first and the second were isolated from a heat-killed culture of virulent human-type tubercle bacilli, strain Nakano, 8 weeks old on Sauton's medium, according to the method of Boquet and Nègre (*1*) and that of Anderson (*2*). The third sample was isolated according to the former method from a culture of attenuated bovine-type tubercle bacilli, 6 weeks old on the same medium and killed by immersion in acetone. All three samples were purified by treatment with boiling acetone.

In experiments parallel to those with the phosphatide samples, use was made of three samples of tuberculin polysaccharide, prepared according to the conventional method of previous deproteination with trichloroacetic acid followed by precipitation with methanol. The first polysaccaride sample was prepared from an unheated culture filtrate of the strain Nakano, 8 weeks old on Sauton's medium. This is a fraction precipitable at 90 percent methanol, after removal of a fraction precipitable at 30 percent methanol. The second and third samples were likewise isolated from an unheated culture filtrate, of the strain $H_{37}Rv$, of the same age on the same medium. These are fractions precipitable at 80 percent and 95 percent methanol, respectively, after removal of a fraction precipitable at 50 percent methanol. For comparison, use was also made of a sample of old tuberculin prepared from a Sauton culture filtrate of human-type tubercle bacilli, strain Aoyama B.

The nitrogen and phosphorus contents of the antigens (in percentages) were as follows: 0.29 and 2.60 for phosphatide sample 1; 0.15 and 1.97 for phosphatide sample 2; 1.47 and 2.68 for phosphatide sample 3; trace and 0.53 for polysaccharide sample 1; 0.22 and 0.15 for polysaccharide sample 2; and 1.00 and 0.20 for polysaccharide sample 3. The Molisch and orcin-HCl reactions were positive for all the antigens, being slightly stronger for the polysaccharide antigens than for the phosphatide antigens. The biuret, Millon, and xanthoprotein reactions were negative for all the antigens.

Tuberculous sera were obtained from rabbits immunized with the following materials prepared from the tubercle bacillus: (i) whole bacilli killed by immersion in acetone (bacilli BA); (ii) bacilli BA successively exhausted with acetone, methanol, and chloroform (residue RC); (iii) residue RC deprived of its bound lipids by treatment with 1 percent HCl alcohol (residue RHA); (iv) living bovine-type tubercle bacilli; (v) heat-killed bacilli of the strain Aoyama B; (vi) living bacilli of the same strain. The first three materials were prepared from the strain Aoyama B. The exhaustion of the bacilli was carried out at temperatures below 40°C.

Sheep erythrocytes sensitized, according to the conventional method, with the foregoing antigens were brought into contact for hemagglutination with serial dilutions of the sera. The results are summarized in Table 1. As is clearly shown in the table, the three phosphatide antigens are capable of sensitizing sheep erythrocytes to hemagglutination in the presence of tuberculous sera experimentally prepared, and without any previous treatment of the cells. Moreover, it is worthy of notice that, while the erythrocytes sensitized with the polysaccharide antigens and the old tuberculin agglutinated in the presence of the

Table 1. Results of tests for hemagglutination reaction. BA, whole bacilli of the human-type strain Aoyama B killed by immersion in acetone; RC, bacilli BA deprived of its free lipids; RHA, resdiue RC deprived of its bound lipids; Pd. 1, 2, and 3, phosphatide samples; Ps. 1, 2, and 3, tuberculin polysaccharide samples. The numbers under column heads for the various samples indicate the maximum, positive dilutions of the sera.

Serum No.	Sheep erythrocytes sensitized with						
	Pd. 1	Pd. 2	Pd. 3	Ps. 1	Ps. 2	Ps. 3	Old tuberculin
			Bacilli BA				
1	1280	1280	2560	640	–	–	–
2	640	640	640	640	160	160	160
3	5120	2560	5120	640	80	160	–
			Residue RC				
4	–	–	–	640	160	320	320
5	–	–	20	1280	320	640	320
6	–	–	–	1280	640	640	640
			Residue RHA				
7	80	–	80	80	–	–	–
8	–	–	20	40	–	–	–
9	40	–	40	80	–	–	–
			Bovine-type tubercle bacilli (living)				
10	1280	640	1280	1280	640	640	640
11	640	640	1280	1280	640	640	640
12	640	160	1280	1280	640	640	320
			Aoyama B (living)				
14	320	320	320	320	160	160	320
15	320	160	320	640	–	640	160
			Aoyama B (killed)				
16	1280	640	1280	640	320	160	160
17	320	320	640	640	320	320	640
			Control				
19	–	–	–	40	–	–	–
20	–	–	–	20	–	–	–

Table 2. Results of the cross inhibition test with serum No. 10, dilution 160. Results of equal tendencies were obtained with serum No. 14. The numbers indicate the maximum inhibiting dilutions of the inhibiting antigens (1 mg/ml). All symbols are defined in Table 1.

Antigen	Pd.1	Pd. 2	Pd. 3	Old tuberculin	Ps. 1	Ps. 2	Ps. 3
Pd. 1	1024	512	512	–	–	–	–
Pd. 2	1024	128	512	–	–		
Pd. 3	1024	2048	2048	–	–	–	–
Old tuberculin	–	–	–	128	1024		
Ps. 1	–	–	–	–	16	16	18
Ps. 2	–	–			128	32	16
Ps. 3	–	–			512	256	256

sera prepared with residue RC (a residue deprived of its free lipids), the cells sensitized with the phosphatide antigens did not. Besides, as is clearly shown in Table 2, the phosphatide hemagglutination reaction was not at all inhibited by the tuberculin polysaccharides, and vice versa (*3*).

The foregoing two facts clearly indicate that, in the animal body, the formation of antibodies to the bacillary phosphatide takes place independently of the formation of polysaccharide antibodies, at least so far as tuberculin polysaccharide is concerned.

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References and Notes

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2. R. J. Anderson, *Fortschr. Chem. org. Naturstoffe* 3, 145 (1939).
3. The mechanism of phosphatide hemagglutination and its clinical meaning in tuberculosis are under investigation.

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Pharmacology and Toxicology of Nicotine with Special Reference to Species Variation

During the brief history of modern pharmacology few chemical entities have received such intensive investigation as nicotine. Langley's demonstration (*1*) in 1889 on the ability of nicotine to inhibit transmissions at synaptic junctions initiated a deluge of literally thousands of articles in the scientific literature.

Several researchers have published on the physiological response of animals to nicotine as an index of neural development in the phylogenetic scale. Greenwood (*2*) reported that the toxic effect of nicotine on invertebrate organisms is determined by the degree of development of the nervous system but that vertebrate animals which have enough in common to stand near each other in the phylogenetic order may react differently to nicotine.

Our interest in this alkaloid results from a screening program designed for the selection of a drug to immobilize wild deer for restocking wildlife conservation areas. The subsequent successful capture and translocation of over two hundred wild white-tailed deer (*Odocileus virginianus*), with nicotine administered by the dart-gun technique previously reported (*3*), warrants the reappraisal of reported toxicity of this chemical.

Gause and Smaragdova (*4*) have compared toxicity of synthetic D-nicotine with natural L-nicotine and have postulated that the variance of toxicity between the two isomers is a function of development of spatially specific receptors at the neuroeffector synapse of voluntary muscles, that it is proportionate to the development of the acetylcholine system.

Pharmacologically, nicotine is considered to be an autonomic active drug with curariformic activity. The classical biphasic action, consisting of evanescent stimulation followed by paralysis of all autonomic ganglia, is generally conceded to be a function of dose, with increasing concentrations involving the central nervous system and myoneural junctions.

The ability of nicotine to induce experimental catatonia, similar to the cataleptoid action of bulbocapnine, was demonstrated by Gutierrez-Noriega (*5*). The maintenance of abnormal or bizzare body positions typical of catalepsy was observed in dogs injected with relatively small doses of nicotine tartrate.

Repeated administration of small or sublethal quantities of the alkaloid, or its salts, results in a rapidly diminishing pharmacological response. Ruppert (*6*) reports that isolobinine and nicotine produce mutual tachyphylaxis in rats and that one-tenth of the effective dose for either of these alkaloids will confer protection against convulsive doses; however, tachyphylaxis is less pronounced in cats and rabbits.

Species variation in ability to eliminate nicotine and in susceptibility to toxic effects was reported by Larson *et al.* (*7*). The median lethal dose, by intravenous injection, was established for cats as 2.0 mg/kg; for dogs, as 5.0 mg/kg; for mice, as 7.1 mg/kg; and for rabbits, as 9.4 mg/kg. However, when nicotine was perfused slowly, intravenously, over an 8-hour period, dogs could tolerate 2.0 times the lethal dose, rabbits 3.3 times, mice 4.6 times, and cats, 10.0 times the lethal dose; this indicates that the ability to metabolize or excrete nicotine is independent of species susceptibility.

Aqueous solutions of chemically pure, natural L-nicotine, unbuffered, of *p*H 8.4, or the equivalent amount of nicotine salt, were administered by intramuscular routes, to all experimental animals. Injection of wild or feral animals was accomplished with the projectile type automatic injecting hypodermic syringe, delivered by a 50-caliber pneumatic rifle (*8*). To avoid development of tachyphylaxis, animals were used only once, or were allowed an interval of not less than 48 hours for eliminating all traces of the drug. The end point for determining paralysis of animals was loss of locomotor function.

The minimal paralytic dose of nicotine and the approximate lethal dose for 14 species are listed in Table 1.

After minimal paralytic doses of nicotine an average of 3 minutes is required for development of symptoms of polyuria, locomotor ataxia, lethargy, and catatonia or flaccid paralysis. Large sublethal amounts of nicotine precipitate severe convulsive seizures, followed by flaccid paralysis and recovery within three hours. Death generally occurs during the convulsive seizure from lethal doses of nicotine; however, monkeys and cattle may succumb to latent effects of minimal lethal doses many hours after their recovery from paralytic effects. Necroscopy studies of these animals reveal pronounced urinary retention, distended ureters, and acute hydronephrosis with petechiasis, indicative of acute uremia. In subparalytic doses, nicotine is an effective emetic in those animals capable of emesis and promotes lethargy or somnolence in all species studied.

The paralytic dose of nicotine administered by intramuscular injection and the approximate lethal dose have been established for 14 species of animals (*9*).

The therapeutic index for nicotine is sufficiently great to allow the use of

Table 1. Paralytic and approximate lethal doses of nicotine, administered by intramuscular injection.

Animal	No. of observations	Minimal effective dose (mg/kg)	Approximate lethal dose (mg/kg)	Therapeutic index
Pigeons	20	5.0	9.0	1.8
Mice	100	5.0	8.0	1.6
Rats	50	10.0	15.0	1.5
Rabbits	30	18.0	30.0	1.6
Guinea pigs	15	8.0	15.0	1.8
Chinchillas	18	10.0	18.0	1.8
Dogs	24	4.5	15.0	3.3
Cats	10	3.5	9.0	2.5
Cattle	38	4.5	9.0	2.0
Horses	6	4.0	8.8	2.2
Swine	4	6.6	> 14.0	not determined
Goats	200	3.0	13.0	4.3
Deer	205	3.0	9.0	3.0
Monkeys	8	4.0	6.0	1.5