Pleuropneumonia-like Organisms Isolated from Bronchopneumonia of Cattle

A bronchopneumonia of varying severity is invariably associated with a disease of cattle referred to as shipping or transit fever (*1*). The disease appears frequently in cattle transported over long distances by ship or rail. That it is a distinct infectious disease is suggested by the constancy of its pathological (*2*) and clinical (*3*) manifestations and the ease with which it is spread to unpredisposed contact cattle.

Bacteriological examinations were conducted on 24 calves ranging in age from 4 to 12 mo, all of which had a severe bronchopneumonia either directly or indirectly related to shipment by rail. The principal bacterial species isolated from the lungs were *Pasteurella hemolytica, P. multocida,* and *Corynebacterium pyogenes*. Details of the bacteriological and pathological findings will be presented in a later report.

With a view to examining for the presence of organisms of the pleuropneumonia group, inocula from the lungs were streaked on "Bacto-PPLO agar" (+1 percent "Bacto-PPLO serum fraction") and "Bacto-tryptose agar" (+20 percent horse serum). On these mediums microscopic colonies were frequently demonstrated in primary cultures, employing the methylene blue-azure stain described by Dienes (*4*). However, in all but two instances subcultures were not successful and it was thought that perhaps the mediums were inadequate. This contention was strengthened by the demonstration in secondary broth cultures of forms that Freundt (*5*) has associated with loss of viability.

With an improved medium, essentially the same as that described by Edward (*6*), two additional isolations were made and maintained through indefinite subcultures without difficulty. On this medium the strains isolated on the PPLO and tryptose agar grew more rapidly and produced larger colonies. The four strains successfully propagated were similar in regard to colonial morphology and all fulfilled Sabin's (*7*) criterions for admission to the pleuropneumonia group of organisms. Characteristic colonies are shown in Fig. 1.

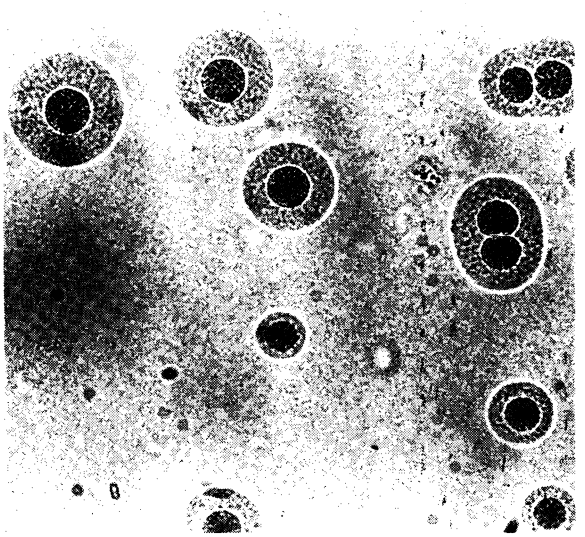

Fig. 1. Colonies of the pleuropneumonia-like organism from bronchopneumonia of cattle (× 120).

That the pleuropneumonia-like organisms isolated are parasitic is suggested by the difficulty experienced in their isolation and propagation. Their recovery from bronchopneumonic lungs of cattle is important if only because of their resemblance to the organism that causes the serious epizootic of cattle, contagious pleuropneumonia.

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The Heparin-like Activity of Certain Inorganic Anions

Phosphotungstate, phosphomolybdate, and silicotungstate have been found to delay the clotting of blood, both *in vitro* and *in vivo*. When injected into rats, they also elicit the appearance in the plasma of lipemia "clearing factor" (*1*), an enzyme that catalyzes the lipolysis of chylomicrons and low-density lipoproteins. This clearing factor appears to be identical with that produced by heparin injection. Both anticoagulant activity and clearing-factor production occur following oral administration of silicotungstate.

The test animals were male Sprague-Dawley rats weighing about 180 g. The usual intravenous dose was 20 mg dissolved in 1 ml of 0.15*M* sodium chloride solution; it was injected either as the acid or as the sodium salt. The usual oral dose of silicotungstate was 200 mg of the salt in 1 ml of water administered by stomach tube in the fasting state. The heparinoid activities were apparent a few minutes after intravenous injection and 20 min after oral administration (Table 1). Lee-White clotting times were prolonged two- to fivefold. Clearing activity was measured by the decrease in optical density of a saline suspension of human chylomicrons incubated at 38°C with 1/10 rat plasma by volume during a period of 1 hr.

The anticogulant activity was inhibited by protamine, both *in vitro* and *in vivo*. Clearing activity was inhibited by protamine and by high sodium chloride concentrations (*2*). During clearing, glycerol was produced. All three salts exhibited metachromatic activity with toluidine blue. In all these respects they resemble heparin.

These findings make it appear likely that the hepar-

Table 1. Clotting time and clearing activity in rats following inorganic salts.

Drug	Dose (mg)	Route	Clotting time (min) Before prot.	Clotting time (min) After prot.*	Clearing activity (o.d. units) Before prot.	Clearing activity (o.d. units) After prot.*	
None			< 4		< 0.015		
Sodium phosphotungstate	20	i.v.	9	2	.11	0.02	
	20	i.v.	13	3	.09	.02	
	20	i.v.			.09	plasma pre-	0.15*M* NaCl
					.03	incubated for	0.76*M* NaCl
					.01	2 hr in	1.37*M* NaCl
Sodium silicotungstate	200	oral	6		.07		
	200	oral	10		.07		

* Immediately after first bleeding, rat received 3 mg protamine and was bled again 3 min later.

inoid activity of a serum mucoprotein precipitated with phosphotungstic acid, as reported by Greenspan (*3*), is an artifact. Samples of phosphotungstate-precipitated mucoprotein prepared by us from bovine serum according to his method contained appreciable amounts of ash. Qualitative analysis indicated the presence of tungsten in these preparations.

The number of substances having heparin-like activity is thus extended to include inorganic compounds. These compounds resemble heparin and other heparinoids in having a relatively high molecular weight and negative charge but differ from them in the absence of carbohydrate and sulfur. Detailed data and additional studies will be reported elsewhere.

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Rapid Separation of Diamond from Other Forms of Carbon

A rapid method for the separation of diamond from other materials, especially other forms of carbon, was desired. It was also desired that the method be quantitative—that is, no loss of diamond.

A number of preliminary experiments were made with various acids, salts, oxidants, and fluxes, but the most effective method found was preferential oxidation with catalyzed perchloric acid.

Approximately 0.1-g samples of the materials listed in the following paragraph were used. Samples were treated as follows: (i) fumed to dryness with red nitric acid; (ii) oxidized with 10 to 20 ml of 60-percent perchloric acid; catalyzed with approximately 0.1-g of ammonium metavanadate at 200°C (generally 30 min); (iii) diluted with water and the insoluble vanadium oxides reduced with an excess of hydroxylamine hydrochloride; (iv) washed, centrifuged, dried, and weighed any diamond residue.

The following materials were treated by the aforedescribed method: (i) carbon blacks—Thermax, Shaw, P33, Dag 154, Spheron 6, and Spheron 9; (ii) graphites—Dixon 200-09 and UCC grade SP2 spectrographic in the form of rods, chunks, and 60-mesh powder; (iii) diamond—0.1 g (approximately) macles and 4000-mesh dust. Materials under (i) and (ii) were completely oxidized in approximately 30 min in most cases. However, some coarse graphites took several hours. Oxidation without catalyst took approximately 10 times longer. Diamond was not attacked in 5 to 6 hr as judged by weighing (± 0.1 mg) and optical examination.

Perchloric acid has been used by others to remove graphite from diamond (*1*). Catalysts have been used with perchloric acid as an oxidant for carbonaceous materials (*2*). Thus the method outlined is an application of these methods. It has been found to be the most convenient and effective method of those investigated.

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