SEROLOGICAL STUDIES ON *PASTEURELLA MULTOCIDA*, ESPECIALLY ON O-ANTIGENIC ANALYSIS OF THE ORGANISM

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Introduction

Previous studies have shown that serology has played an important role with regard to analyzing the etiology and epidemiology of salmonellosis and colibacillosis. Another important key has been the discovery of the correlation between the serotype and host specificity of *Pasteurella multocida* as a part of our research into these organisms.

Recently, the serological classification according to the serotype of *Salmonella* which was established by Kauffmann *et al.* has been widely criticized and there are many scientists who feel that bacteriological classification should be made according to advanced molecular biology or the Adansonian principles of classification advocated by Sneath.[30] These scientists tend to consider the former classification as diagnostic or enumerative and belonging to classical methodology. However, in the establishment of the serotype of enteric bacteria (Family Enterobacteriaceae) for example, the significance of the actual analysis of epidemiology and prophylaxis must also be considered individually along with advanced methods of microbiology. For this reason, the data reported in the literature regarding the serological classification of *P. multocida* have been reviewed and used along with the results of observations made in the present study.

History of serological studies on *P. multocida*

There are many ambiguous points in the various initial reports concerning the isolation of *P. multocida* and at the end of the 19th century, Pasteur had isolated the organism from chickens infected with fowl cholera and used the avirulent strain of the bacteria as a vaccine. In the first few years of the 20th century, the organisms were isolated in various animals with sepsis and identified although the relations between the various strains were not as yet clear. The concept of Lignieres (1900)[16] concerning the taxonomy of these groups of bacteria was generally accepted and the so-called zoological classification in which the names referred to the animal of origin was established. In this system, the strain recovered from cows was called *P. boviseptica*, that from birds *P. aviseptica* and that from sheep *P. oviseptica*. However, this classification system was not fully accepted at that time and there was some opposition based mainly on the fact that the relationship between the serotype and each species had not been clarified. There were reports of strains with no serological relation in strains from the same species whereas common antigenic relationships between different strains from different animals could be demonstrated.[11] Even in these cases, however, there was no consistent typing method among the various researchers and since the serological reaction was carried out by various methods, the work ended with no standardized means of measurement.[17,31]

In 1939, Rosenbusch & Merchant[29] investigated about 30 strains of a group of similar organisms obtained from various species of animals, mainly with respect to their biochemical properties. They found that the differences which should exist between the various strains were not present and they suggested that the groups which had been divided under the zoological classification be reclassified together as *P. multocida*. This proposal was accepted in Bergy's Manual of Determinative Bacteriology 6th ed. (1948) and is still in use today.

Rosenbusch *et al.* also noted the existence of two biotypes (I and II) in *P. multocida* from the results of two or three sugar fermentations and serological reactions but this has been of little importance concerning the classification. On the basis of the same interpretation as that of

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Rosenbusch *et al.*, Topley & Wilson [32] in England named this group of organisms *Pasteurella septica*.

On the basis of this work, the organisms formerly classified under the zoological system were grouped together as *P. multocida* without any clarification of the relationship between the serotype and epizootiology of the organisms. One reason for this was that the organisms easily dissociated various colonial forms after isolation and since there were complex changes in the antigenicity and serological reaction as well as colonial variation, it was difficult to determine the phase of variation which was standard for the serological reaction.

In addition to this international classification, Ochi[24] from Japan developed his own classification while working in a laboratory in Korea from 1930 to 1935. He also objected to Lignieres' zoological classification, divided *P. multocida* into four types and emphasized the relation between each of these types and the host specificity for various types of animals.

These four types were tentatively named types A, B, C and D. Type A was pathogenic only in chickens and was a causal agent of fowl cholera. Type B was not pathogenic in chickens but had host specificity for cattle, sheep and swine and was the pathogen for hemorrhagic septicemia in these animals. Types C and D could not cause hemorrhagic septicemia in any animal, but were secondary invaders in humans and animals; they were related to pneumonia and local infections, and had a comparatively low virulence.[24]

Several years after Rosenbusch *et al.* had named the organisms *P. multocida* in 1939, Carter[4] reported the existence of a capsular substance in a fresh culture of the organism. This was rather rapidly lost when a sub-culture was made on artificial media. He called the former the fluorescent type (later changed to iridescent type) and the latter the blue type. He extracted this capsular substance (K antigen) in saline solution and subjected it to the precipitation reaction for antigens. The organisms were divided into four specific serotypes in accordance with this reaction. He then adsorbed the K antigen on a human O erythrocyte and attempted typing using the hemagglutination reaction.[5] The concept of serological reaction using K antigen was inferred in the report by Hoffenreich[14] in 1928 under the title "Kapselsubstanz aus *Bacillus avisepticus*." The contents of this report are very complete even though it consists of only 2 pages. The presence of K antigen in *P. multocida* was clearly shown and it was stated that the K antigen was a polysaccharide. When rabbits were inoculated with a saline extract, the specific K antibody was produced.

Starting with *P. multocida* as the initial large group, Carter carried out the serological typing and classified the types as A, B, C and D based on the capsular antigens. In 1947, shortly before the work of Carter, Roberts[28] used 20 strains of *Pasteurella* obtained from various animals to perform cross protection tests with mice. From the results of the tests, he divided the organisms into four groups named types I, II, III and IV. Carter performed comparative research concerning the relation between his own serological classification and Roberts' classification, and showed that his types A, B, C and D corresponded to Roberts' types II, I, III and IV. It was tentatively concluded that the protection substance of the *Pasteurella* organism obtained by Roberts was identical with the K antigen.[5]

Therefore, Carter's serological classification of the *Pasteurella* organism is now widely used internationally as the most reliable system and it was included in Bergy's Manual 7th ed. (1957).

There should be some relation between the serotype of Carter's K antigen and the animal of origin or the process of the disease. Carter had investigated the capsular type of *Pasteurella* from various types of animals several times between 1952 and 1959.[6,7] In 1960, Carter & Bain[10] published a review concerning *P. multocida*. As can be seen in Table 1 taken from this review, more than 800 strains from 20 different types of animals have been typed according to capsular antigens. The relation between the serotype and the process of disease is not given in this table but the authors outline the relation in the review. They state that types A and D are the most widely distributed geographically. A large part of the *P. multocida* were isolated in the northern hemisphere, especially in Canada and the United States. The sources also vary widely and include fowl cholera, septicemia or pneumonia of various animals and local wounds in humans. All of the organisms belonging to types A and D usually kill chickens when given in very small doses. Also

type B organisms are mainly isolated from hemorrhagic septicemia in cattle and cattle die when inoculated with 20,000 or less of these organisms subcutaneously. The Carter's type C has been reported as often originating in the upper regions of the throat of cats and dogs but since 1955, a rough variation has arisen in the standard strain of type C and the distribution has also become very limited. For these reasons, type C has been discarded (refer to Table 1). As is also evident from Table 1, about 35% of all the organisms listed by Carter cannot be typed. This may be due to the fact that many of the organisms sent to him after isolation had already lost their capsules. This means that many organisms which are not clearly typed will be encountered when depending only on the K antigen.

Table 1 Relation between serological type of *P. multocida* and animal host (Carter and Bain, 1960)[10]

Animal host	Type (Capsular)			Not typable
	A	B	D	
Buffalo	2	13	0	0
Caribou	3	0	0	0
Cat	3	0	1	27
Cattle	88	23	6	47
Chinchilla	0	0	0	7
Chipmunck	0	0	0	1
Deer	0	1	1	0
Dog	0	0	3	14
Fowl	56	0	6	125
Goat	0	0	1	1
Guinea pig	1	0	2	1
Horse	0	1	1	1
Man	20	0	11	16
Mink	3	0	1	10
Monkey	1	0	0	0
Mouse	0	0	2	3
Muskrat	1	0	0	2
Pig	157	3	118	19
Rabbit	11	0	3	13
Sheep	11	1	9	13
Total	357	42	165	300

As was mentioned previously, all strains belonging to type A are highly pathogenic in fowl and have the ability to protect the host against the various strains by means of the K antigen. Carter has also reported that intracellular antigens consist of complex substances and these substances are common to all capsular type strains. He concluded that the basis for serotyping and epizootiological observations of *P. multocida* tend to emphasize the importance of the K antigen of the organisms.[10]

Progress of our studies on serological classification of *P. multocida*

Since 1958, the author has carried out serological studies and made epizootiological observations on *P. multocida* from Japan and various types of organisms obtained from animals distributed throughout Southeast Asia.[22] All this work has been based on the Carter's method.

However, one problem has arisen; six strains from a fresh culture obtained from swine pneumonia and sent to us by Professor Liu of Taiwan University had remarkably different pathogenicities in respect to chickens despite of the fact that they all belonged to Carter's type A. Three of these strains always killed three-month old chickens within 48 hours when administered in small amounts (about 300 organisms). However, the other three strains did not kill the chickens even when the animals were inoculated with more than two million organisms. The same results were obtained by repeated experiments.

From these results, it was evident that some of Carter's type A organisms isolated from swine pneumonia do cause fowl cholera in chickens and some do not. From the viewpoint of pathogenicity for chickens, there are therefore two completely opposite types among the Carter's type A organisms. In order to explain this phenomenon the author studied the somatic antigen (0 antigen) of *P. multocida*.

The organisms were collected from various types of animals all over the world. As a result about 100 strains were obtained from different countries including Canada, France, Egypt, the

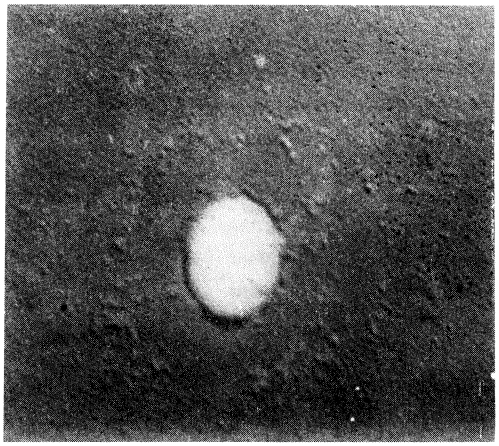
Table 2 Agglutination tests between antisera prepared with formalinized antigen and the homologous strain treated by various means[20)]

Antigen		Antiserum prepared with 0.3% formalinized antigen		
		3397	656	P8497
3397	Capsular type A (mucoid type)			
	Formalinized	0		
	75°C, 1 hr	0		
	100°C, 1 hr	0		
	121°C, 1 hr	160*1		
	Alcohol, 37°	0		
	N-HCl, 37°	320*2		
656	Capsular type B (Blue type)			
	Formalinized		1,280*1	
	75°C, 1 hr		640*1	
	100°C, 1 hr		320*1	
	121°C, 2 hrs		320*1	
	Alcohol, 37°C		20*1	
	N-HCl, 27°C		320*2	
P8497	Capsular type D (Fluorescent)			
	Formalinized			80*3
	75°C, 1 hr			160*1
	100°C, 1 hr			320*1
	121°C, 2 hrs			320*1
	Alcohol, 37°C			0
	N-HCl, 37°C			640*2

*1 Floccular)
*2 Granular) agglutination
*3 Disc-shaped)

Philippines, Burma, Viet-Nam and Taiwan. Experiments were then performed using these strains.

As was mentioned previously, *P. multocida* rapidly dissociates after initial isolation when subcultured on artificial media. Since the capsule form exists in fresh cultures, the colony shows a beautiful fluorescent color under oblique light (iridescent type). After subculturing, the capsules are lost and the colony becomes colorless and transparent (blue type). When the colony contains mucus, the fluorescent colors weaken (mucoid type). The colony form that is flat with notches around the margin is known as the rough type (R type). The presence of the K antigen can be confirmed by the capsular swelling reaction of India ink by the antiserum but we succeeded in obtaining a purple color using the Møller's method.[18] Carter tentatively stated that the blue type is the R type and designated it as R^S. However, this indicates a loss variant of the K antigen and it is difficult to think of the somatic antigen as having changed to a rough antigen. Carter's mode of colonial variation for *P. multocida* is based on that of *Diplococcus pneumoniae* developed by Griffith.[12] The author considered, however, that the phenomenon of so-called S-R variation of *P. multocida* would coincide with that of V-W variation of *Salmonella typhosa* or other enteric bacteria.



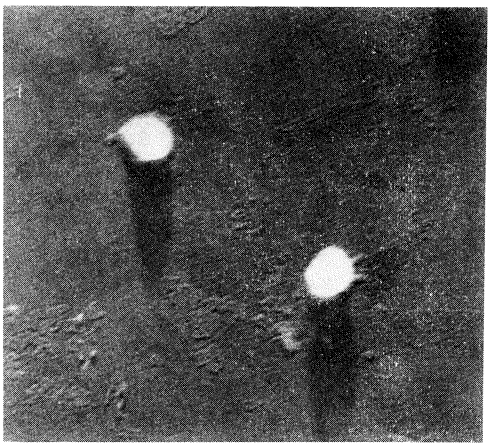
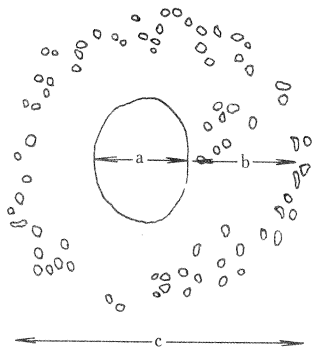


Fig. 2 ***P. multocida*** **Strain 3397 (1:A) Treated with 1NHCl, X5,000**



a: Bacterial body (soma)
b: Capsule (broken by electron)
c: Whole cell

Fig. 1 ***P. multocida*** **Strain 3397 (1:A) X5,000, Whole cell**

If such *P. multocida* is obtained from one original culture and the various variants arise, the antiserum obtained will naturally contain variable antibodies. From the serum reaction between this antiserum and the antigens from the same organisms at the various levels of variation, it is easy to imagine that results will be difficult to interprete. In the 1920s when it was not clear that the form of variation of these organisms was concerned with K and M antigens, there were attempts to find the relation between the *P. multocida* process of disease and the type of animal from which the organisms were obtained but naturally no definite conclusion was reached.

At first we chose the iridescent type, blue type and mucoid type from among the organisms collected and made several antigens using various types of treatment. Agglutination tests were performed in each of the homologous antisera.[20] The results were as given in Table 2.

As can be seen from this Table, different results are obtained for the same type of organism with formalin and boiled antigen depending on the antigen with variations in steps. Granular agglutination characteristics of somatic antigens, however, were obtained when the surface substance was destroyed by 1_N HC1. Even at a temperature of 121°C, the same agglutination was obtained but the agglutinability was floccular and lacked 0 agglutination stability. Then the serological properties of these

Table 3 Relation between *Pasteurella* capsule and somatic serotypes and host animals

O group	Capsule	Serotype	Process of disease	Animals	Strain examined
1	A	1 : A	Pneumonia	Swine	9
			Septicemia	Mouse	2
	D	1 : D	Pneumonia	Swine	1
	–*	1 : –	Pneumonia	Swine	1
				Sheep	2
				Cattle	2
2	D	2 : D	Pneumonia	Swine	12
3	A	3 : A	Pneumonia	Swine	3
	D	3 : D	Pneumonia	Cat	1
4	D	4 : D	Pneumonia	Swine	2
			Pneumonia	Sheep	2
5	A	5 : A	Fowl cholera	Fowl	13
			Pneumonia	Swine	3
	–	5 : –	Fowl cholera	Fowl	21
			Pneumonia	Swine	1
			Local wound	Man	1
6	B	6 : B	Hem. Sept.	Cattle	6
	E	6 : E	Hem. Sept.	Cattle	1
	–	6 : –	Hem. Sept.	Cattle	10
7	A	7 : A	Septicemia	Cattle	5
	–	7 : –	Septicemia	Cattle	2
8	A	8 : A	Fowl cholera	Fowl	1
9	A	9 : A	Fowl cholera	Fowl	7
10	D	10 : D	Pneumonia	Swine	1
11	B	11 : B	Local wound	Cattle	1

* No capsule

Hem. Sept.: Hemorrhagic septicemia

organisms were investigated. If the organisms employed had not been of the R type, it would have been possible to obtain clear 0 agglutination selectively with 1_N HCl treatment regardless of the process of colonial variation. From the results of absorption tests among the strains, it was confirmed that the 0 antigens of *P. multocida* can be classified as common or specific.[20]

When organisms with capsules and organisms treated with 1_N HCl were examined under an electron microscope, both showed a spherical shape but the latter (Fig. 2) characteristically had been reduced to about one-fifth the size of the former (Fig. 1).

Since a considerable amount of nucleic acid and saccharide had been included in the supernatant treated with HCl, the surface substance and various types of intracellular substances were dissolved in the solvent during the treatment, and this is considered to be the reason why the organisms were reduced in size.

The question arises as to what type of substance remains as the somatic antigen after the HCl treatment. In a personal communication, Bain stated that it consists of a part of the cell wall substance and therefore, it is probably appropriate to call such a somatic antigen a cell wall antigen. Therefore, it is assumed that the substance is a lipopolysaccharide combined with a protein. Further investigations are needed in the future to determine its exact composition.

In any case, the 0 antigen of these organisms is a combination of several factors and resembles the organization of the 0 group of *Escherichia coli*. Therefore it is appropriate to call the somatic antigen of *P. multocida* "0 group" rather than "0 type".[21] Even among strains of the same 0 group, there is no complete agreement about the antigens. For example, it is known that in 0 groups 1 and 6 there are several 0 subgroups.[21]

Subsequently, 50 strains of *Pasteurella* organisms isolated in the United States were added and when they were serotyped, it was found that 12 0 groups were present. About 150 strains collected by combination with Carter's capsular antigens were divided into more than 15 serotypes.[22] These results are shown in Table 3.

Table 4 Relation between Carter's capsular and O group[23]

Carter's capsular group	O group
A	1
	3
	5*
	7
	8*
	9*
B	6**
	11
D	1
	3
	4
	5
	10
	12
E	6**

* Pathogen of fowl cholera
** Pathogen of hemorrhagic septicemia

As is evident from this Table, the six 0 groups belong to the Carter's type A, two 0 groups to type B, six 0 groups to type D and one 0 group to type E. Table 4 shows an arrangement of these relations.

Pathogenicity and etiology of *P. multocida* with special reference to haemorrhagic septicaemia and fowl cholera

1 Fowl

When the serotype of the *Pasteurella* organisms is expressed as a combination of the 0 group and K antigen, the relation between this combination and the type of disease in various types of animals comes into question. The author will discuss two or three aspects connected with this problem.

The first concerns fowl pathogenicity. Experimentally, the only organisms capable of producing fowl cholera (hemorrhagic septicemia in fowl) are those with certain 0 groups among the organisms belonging to Carter's type A. These include 5:A and 8:A. Experiments have shown that other types, i.e. 1:A, 3:A, 7:A etc., have no pathogenicity for fowl, and these types have naturally not been found in field cases of fowl cholera up to now.

In the United States, a disease has been observed that clearly differs from typical fowl cholera (hemorrhagic septicemia in fowl) and from which 2:D, ?:D had often been isolated. The disease is called chronic fowl cholera. It has a very low mortality and pathological changes such as sinusitis are limited. Therefore, it is advisable that this type of fowl cholera should be called fowl pasteurellosis rather than chronic fowl cholera.

In 1962, Heddleston[13] in the United States used a polyvalent vaccine for fowl cholera. This vaccine was made from strain P1059 and strain 70-X originating from fowl cholera in turkeys and chickens in the United States. Heddleston also pointed out that two types of organisms which differ immunologically cause fowl cholera. Both of these organisms are inoculated in chickens and the challenge of the heterologous strains cannot be resisted. When these two strains are serotyped, both belong to the Carter's capsular type A but 0 group 8 is present in P1059 and 0 group 5 in 70-X. This indicates that it will be possible for new serotypes which cause the fowl cholera in various countries to be added.

The important point here is that since 5:A and 8:A are of the same capsular type, and fowl cholera can be produced in the three-month chickens even with very few organisms, the 8:A vaccine will not protect against 5:A infection and conversely the 5:A vaccine will not protect against 8:A infection.

Therefore, it is clear from these results that the capsule antigen is not sufficient to protect the host. The causal agents of fowl cholera are organisms of at least two serotypes and even if the chicken is immunized with one serotype, there will be no protection against fowl cholera infection caused by the other serotypes.

Murata *et al.*[19] have investigated the pathogenicity and protective ability of types 1:A and 5:A of *P. multocida* in chickens and mice. They found that only type 5:A was pathogenic in chickens* and both types were pathogenic in mice. Naturally, chickens immunized with strain of 1:A were not protected against a challenge of the strain of 5:A and the reverse was also true for mice.

When the iridescent and blue types of colonial formation in certain strains are compared, however, the pathogenicity in the same host is naturally stronger in the case of the former. This can also be explained by stating that the virulence of *Salmonella typhosa* containing Vi antigen has more pathogenicity compared with the Vi loss variant of the same organism.

From these results, it is evident that the presence of 0 groups cannot be overlooked when investigating the relation between the serotype and etiology of *Pasteurella* organisms. Therefore it has been suggested that the Carter's type A would be more correctly expressed as group A.[21] This

* Pathological findings of fowl cholera which are also histopathologically characteristic were observed in the infected chickens.

would indicate that the serotype of *P. multocida* was actually a combination of the capsular antigen and 0 group.

In other words, Carter's types correspond to the group of *Salmonella*. The Carter's type D is exactly the same as the group. For example, the *Salmonella* D group includes *S. typhosa* (9, 12, Vi: d:-) which is a causal agent of typhoid in humans, *S. enteritidis* (9, 12: g, m:-) and *S. gallinarum-pullorum* (1, 9, 12:-). Although these belong to the same group, their pathogenicity varies with the host. The serotype, however, is finally determined by the combination of 0 and H antigens and the relationship between the host and pathogenicity becomes clear. Thus, as can be seen in Table 3, 5:A is often isolated in Taiwan from cases of swine pneumonia. In the case of 5:A, the type of disease varies with the kind of animals. For example, this organism causes fowl cholera in chickens but it will not produce hemorrhagic septicemia in swine. This is very similar to the case where *S. enteritidis* causes typhoid in mice but in humans produces only enteritis. For this reason, Carter[8] changed his types A, B, D and E to groups A, B, D, and E.

2 Cattle

Both Carter's and our results show that strains belonging to Carter's group B (Robert's type I) consist only of serotype 6:B when all of the group B strains were obtained from hemorrhagic septicemia in cattle. However, Bain *et al.,* have also reported another type of organism which belongs to Carter's type B.[2] They found that one *P. multocida* (Australian type) was isolated from the local wound of cattle in Australia. Although the organism was identified as belonging to Carter's group B, it did not cause hemorrhagic septicemia experimentally in cattle and clearly differed with respect to the pathogenicity from the group B organisms (Asian type) isolated in Southeast Asia where they produced hemorrhagic septicemia experimentally in cattle. When comparing the capsular substance in both organisms, it was found that there were no serological or biochemical differences. Bain & Knox,[3] Knox & Bain[15] performed phenol extraction on the intracellular substance using the method of Westphal *et al.* and analyzed the lipopolysaccharide obtained. They found that there was three times more aldoheptose in the Asian type than in the Australian type.[3,15] From these results, Bain concluded that the existing capsular type was inadequate in explaining the relationship between epizootiology and serotype.

The author also investigated the distribution of Bain's Australian type (strain 989) as well as its serotyping. It was confirmed that the strain 989 had a new somatic antigen (0 group 11) which bears no relation to that of 0 group 6.[23] Even though the capsular antigen is of the Carter's group B, it does not belong to the 0 group 6 (at present) and the organism is not a causal agent of hemorrhagic septicemia in cattle. This is the same as the previously described case where type 1:A and 3:A did not produce fowl cholera while type 5:A did. When a mouse immunized with the Australian type (11:B) was challenged with the Asian type (6:B), there was no protection as also noted by Bain.

When Dr. Perreau from Chad in Central Africa visited our laboratory during a trip to Japan in 1960, he said that *Pasteurella* from hemorrhagic septicemia of cattle in Central Africa were not the same as the Carter's Group B. Later he sent us three strains of *P. multocida* from cattle in Chad but these had already lost their capsular form and were of the blue type. However, all of these organisms belonged to 0 group 6 and were typed as 6:-. Carter also investigated strains from hemorrhagic septicemia of cattle in Congo. He confirmed that these organisms had capsules which differed from those of group B and he designated them as a new group, group E.[9] Subsequently, the author often received organisms of group E from Bain and found that they belonged to 0 group 6.[23] In a personal communication, Bain considered that groups E and B protected mice against infection reciprocally. Therefore, even if the K antigen differs, there will be the same pathogenicity in certain hosts if the 0 group is the same and it is also evident that both strains protect against infection. This is summarized in Table 5.

Table 5 Relationship between serotype and pasteurellosis[23]

Carter's capsular group	O group	Sero-type		Process of disease	Animals	Immunization
A	5	5 : A	Aviseptica group	Fowl cholera	Chicken	Do not protect fowl or mice with heterologous strains
	8	8 : A			Duck	
	9	9 : A				
B	6*1	6 : B	Boviseptica group	Hemorrhagic septicemia	Cattle	Protect cattle *2 or mice with heterologous strains
E	6	6 : E				
A	1*3	1 : A				
	3	3 : A				
	7	7 : A				
B	11	11 : B		Pneumonia, Local Wound, Secondary infection	Various animals and man	Do not protect mice with heterologous strains
D	1	1 : D				
	2	2 : D				
	3	3 : D				
	4	4 : D				
	10	10 : D				
	12	12 : D	(Provisional)			

*1 Subgroup present in 0 group 6.
*2 Protection tests are not complete with some strains, i.e. various protection degrees are seen in some 0 subgroups in 0 group 6.
*3 Subgroups present in 0 group 1.

Protective substances of *P. multocida*

The virulence of *P. multocida* is quickly decreased by subculturing the organism on artificial media due to loss of capsular substance. Roberts has reported from the results of reciprocal protection tests by means of passive immunization of mice that the capsule antigen is the main protective substance of *P. multocida*.[28] On the basis of these results, Carter *et al.*[10] also stated that the vaccine alone from a fresh culture insures protection. However, Bain has reported that a fresh culture of *P. multocida* 6:B which causes hemorrhagic septicemia in cattle has a kind of masked antigen which differs from the capsular antigen according to Carter, and he called it phase I.[1] When he made a vaccine from organisms of group 6:B which had lost phase I, the host was not given complete protection even when the capsule was present. However, at the present time, there is no serological proof concerning phase I. In the work reported up to now, it is considered that the somatic (0) antigen has considerable influence on the protective substance of these organisms when the 0 group differs even though capsular type in *P. multocida* is identical. This is because there have been no results which did not corroborate the reciprocal protection tests.

Recently, Prince & Smith[25-27] treated 6:B organisms isolated from hemorrhagic septicemia of cattle by various methods and found antigenic substances with about 20 different types of immunogenicity by means of Ouchterlony's gel-precipitation and immunoelectrophoresis. They concluded that the most important antigens related to the serotype and protective agent were the β antigen in the capsule substance and the γ antigen (the 0 group of Namioka *et al.*) which can be

considered as a somatic antigen and is the main component of the HC1-treated antigen. Thus β (a part of the capsular substance) and the γ (somatic antigen) are the specific antigens which determine the serotype. The other substances are common to *P. multocida* and have almost no importance as protective agents.

The disease which is customarily known in Japan as swine plague (Schweineseuche) has at present nothing which indicates that it is a porcine disease. In Japan, when *P. multocida* is isolated from swine pneumonia, it is tentatively called swine plague. In a report published in the 1930s, Ochi[24] stated that inoculation of cattle, swine and sheep with small amounts of type B organisms caused hemorrhagic septicemia experimentally in these animals.[24] However, a disease of swine which corresponds to hemorrhagic septicemia of cattle is now very rare throughout the world.

Carter & Bain[10] experimentally produced cattle hemorrhagic septicemia with certain organisms recovered from cattle. They found that 10,000 times the normal amount of organisms which kill cattle were necessary to kill the swine. When limited to hemorrhagic septicemia, they doubted that there was a host specific serotype present in swine.[10] The *Pasteurellae* from swine pneumonia in Japan are limited at present to the 1:A and 2:D serotypes, and these will not cause hemorrhagic septicemia experimentally in either swine or chickens. Even if *Pasteurella* is isolated from swine pneumonia, it cannot be definitely concluded that it is the primary causal agent. When thinking about the etiology of swine pneumonia in Japan, it is essential to give due consideration to *Mycoplasma hyopneumoniae* and certain viruses.

In the past, *Pasteurella* organisms were thought to be the causal agents of shipping fever in cattle, but now it has been verified experimentally that the infection is due to parainfluenza virus and *Pasteurellae* are only secondary invaders.

Therefore, even though *Pasteurella* organisms have been isolated from swine pneumonia, care is needed when concluding that they are the causal agent and there are objections to calling this disease swine plague.

At the present time, the names swine plague and chronic fowl cholera have vague meanings. Therefore, more research is needed into the relation of the serotype in pasteurellosis of various types of animals.

Summary

Serological studies on *Pasteurella multocida* were started about 50 years ago in order to correlate serotypes with the zoological classification of the organism. In 1947, Roberts studied 37 *Pasteurella* cultures and classified them into four types (1-4) by cross-protection experiments on mice. After the work of Roberts, Carter proved that the capsule antigens of *P. multocida* were divided into four serological types, A, B, C and D (later, type E was added) by means of precipitation (1952) and hemagglutination test (1955).

In addition to the capsular typing by Carter, Namioka *et al.* carried out 0-antigenic analysis (1961) and identified 11 0-groups (1964). The 0-antigen of these organisms is a combination of several factors and resembles the organisation of the 0-group of *Escherichia coli*. Treating the organism with 1 N HC1 is recommended for removing capsular substance from the organisms and preparing available 0-antigen. With the combination of capsular (K) group and 0-groups, there are 15 serotypes in *P. multocida* (1A, 5A, 2D 6B, cE, etc.): six 0-groups belong to Carter's group A, two 0-groups to group B, six 0-groups to group D and one 0-group to group E.

Experimentally, the only organisms capable of producing fowl cholera are those with certain 0-group (0-5 and 8) among the organisms belonging to Carter's group A. These include 5A and 8A; experiments have shown that other types (e.g., 1A, 3A, 7A) have not produced fowl cholera up to now. Naturally, chickens immunized with strains of 8A were not protected against a challenge of the strain of 5A, and the reverse was also true for mice. These findings indicate that the 0-groups of the organisms play an important role in elucidating the host-parasite relationship in pasteurellosis. Therefore, it might be wise to represent the serotype of *Pasteurella* organisms with K- and 0-antigens.

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Discussion

Joseph P.G. (Malaysia): Have you had any isolation of indol negative, non hemolytic *Pasteurella gallinarum* from poultry and, if so, could the organisms be typed according to the procedure you described.

Answer: I have not had the opportunity of isolating *P. gallinarum* from chickens. I believe that there is no serological relationship between *P. gallinarum* and *P. multocida*. I think that for the typing of *P. gallinarum* the procedure applied to *P. multocida* can be adopted.

Sato S. (Japan): Do you have many cases of *P. gallinarum* in your country? In Japan, this organism has been isolated from chickens with chronic respiratory disease at the rate of about 20% (Sato *et al.* 1972)

Answer: Joseph P.G. (Malaysia): In Malaysia, the acute form of fowl cholera is rare (2,3 outbreaks). However, *Pasteurella gallinarum*, although not common, is isolated occasionally from birds which have died of chronic fowl cholera.