CHARACTERS OF STAPHYLOCOCCI ISOLATED FROM DEAD CHICK EMBRYOS AND FROM PATHOLOGICAL CONDITIONS IN CHICKENS

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INTRODUCTION

A number of reports have stated that staphylococci have been isolated frequently from dead chick embryos. In Japan, HAMADA obtained staphylococcal cultures from 2 per cent of yolk materials of a total of 795 dead germs and dead-in-shell chicks. In India, SINGH isolated 65 cultures of staphylococci from 300 "dead in shell" chick embryos. However, those two reports did not deal with biological characteristics of the staphylococci, though they indicated that staphylococci might be of importance in the etiology of deaths of chick embryos.

GORDON et al. pointed out that staphylococci and fecal-type organisms may be responsible for either a greater number of outbreaks of infected yolk sacs or of decreased hatchability than has hitherto been suspected. Recently, HARRY reported that the death of embryos and chicks resulted from an infection of the yolk by a number of bacteria of types which are frequently present in the alimentary tract, and on the skin of the hen. According to his opinion, yolk infection in chicks is usually initiated by non-pathogenic bacteria, which are isolated from sites other than the yolk and possess enzymes capable of degrading the yolk protein complex, or occasionally by toxigenic bacteria capable of stimulating the production of an inflammatory exudate from the yolk sac lining. The same conditions may exist in yolk infection in embryos. Moreover, he reported outbreaks in which *Staphylococcus aureus* of human origin appeared to be the cause of the yolk infection. SMITH[19] stated that, although staphylococci have been considered by some workers to be the cause of omphalitis or mushy chick disease in baby chicks, further proof is needed since some of the cultures isolated from chicks which died from this disease have been coagulase-negative. Uuder these circumstances, it seems important that the characters of staphylococci isolated from dead chick embryos should be investigated.

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On the other hand, staphylococcal infection in young chickens appears to be a serious problem in broiler production. The disease usually attacks young chickens kept in battery and it is denominated "battery disease" in Japan, and "putrid disease" in Italy[9]. In the present study, characters of staphylococci which were isolated from the above-noted condition of chickens in Japan and in Italy were investigated.

The present paper deals with the frequency with which staphylococci were isolated from embryonating eggs and with the characters of the staphylococcal strains from dead embryos and from diseased chickens.

Materials and Methods

Dead chick embryos Dead-in-shell chick embryos were forwarded from a number of commercial hatcheries in Hokkaido. Most of the embryos were supposed to be dead in the last stage of incubation.

Cultivation of embryos After disinfection of the surface of the shell, a loopful of the yolk was cultivated on B.T.B. lactose agar. This medium was employed to obtain growths of *Enterobacteriaceae* at the same time. After overnight incubation, pure or dominant cultures were preserved for bacteriological examination. However 9 out of 49 staphylococcal strains isolated were lost before examination.

Isolation of staphylococci from samples of chick down Samples of chick down were collected from commercial hatcheries that supplied the chick embryos in Hokkaido. A small amount of the samples was inoculated into nutrient broth. The overnight broth cultures were cultivated on mannitol salt agar. Growths of staphylococci on the media were examined.

Strains from diseased chickens Twelve strains were employed. Among them, 3 were forwarded from Dr. C. Rossi, Italy. Five from 3 outbreaks of infection were supplied by Professor Iwamori of Gifu University. Two were isolated from the hemorrhagic gangrenes of young chickens in Sapporo by the present authors in 1956[15]. The remaining 2 were from adult chickens infected with fowl pox. Seven strains, one human, 2 horse and 4 of bovine origin, were used as controls.

Examination of characteristics of staphylococcal strains Chief biological characteristics were examined by the following methods.

Pigment production: Pigment production on 10% skimmed milk agar was recorded, after materials had been left one day at room temperature following overnight incubation at 37°C. "Yellow" in this study includes pale to golden.

Coagulase test: Two kinds of citrated plasma, rabbit and human, were diluted 2.5 times in saline. A certain volume of 0.5 ml of 24-hour peptone water cultures of the organisms was added into equal volumes of diluted plasma. The mixtures were kept in water-bath at 37°C. Readings were made after 4 hours and the final readings after the mixtures had been left overnight at room temperature.

Hemolysin production: Hemolysin types were determined according to Nakagawa's descriptions[11]. Two or three per cent blood agars prepared from rabbit, sheep and human red cells were employed.

Fermentation of carbohydrates: Modified BARSIEKOW's media inoculated with the organisms were observed for 3 weeks.

Slide-agglutination test Preparation of antisera and procedure of the test were carried on by the method of OEDING[21]. In the present study, unabsorbed serum was employed.

Bacteriophage typing Two sets of phages, the International Series and NAKAGAWA's Bovine phages[13], were employed for typing. The former consists of 20 phages and the latter of 13 which were induced from staphylococcal strains which originated from bovine milk. Details of the two sets are as follows:

The International phages	group I	: 29, 52 A, 52 and 79
	group II	: 3 C, 3 B, 3 A, 55 and 71
	group III	: 70, 42 E, 6, 7, 73, 47, 54, 75, 53 and 77
	group IV	: 42 D
The Bovine phages	group A type A_1	: H 98, 260, 264, 316, 365 and T 90
	type A_2	: 418, 257, H 30 and H 30 a
	group B	: H 131, Y 97 and 883

Phage typing was carried out by means of NAKAGAWA's modification[12] of the method of WILLIAMS and RIPPON, except that three phage concentrations, 1×R.T.D., 10×R.T.D. and 100×R.T.D., were used for routine typing. According to NAKAGAWA[12], the strains which were lysed strongly (++ and CL) by phages at 10×R.T.D. were regarded as typable.

Detection of lysogenic strains Thirty-nine strains isolated from chick embryos and the egg-shell, of which 15 were coagulase-positive and 24 coagulase-negative, were examined for lysogenicity by the cross-culture method of FISK. In this experiment, 61 strains including 29 coagulase producers were employed as indicator strains.

RESULTS

1. Frequency of isolation of staphylococci and other groups of organisms

A total of 3,463 embryos collected from 11 hatcheries were subjected to bacteriological examination. These embryos were derived from 40 hatches. Frequencies with which staphylococci and other groups of bacteria were isolated from the embryos are shown in table 1.

1) Staphylococci Forty-nine embryos gave growths of the organisms. Staphylococci occurred at 18 hatches of hatcheries. Yolk materials of 337 embryonating eggs from 2 hatcheries and the egg-white on the surface of their embryos were together examined. Cultures of staphylococcus were obtained from the egg-white of 7 embryonating eggs. In all excepting one of the above 7 eggs, both the yolk and the egg-white showed staphylococcal growths at the same time.

2) Other groups of organisms from chick embryos A total of 154 embryos yielded several types of Salmonella. These types included *S. thompson* (0.2%), *S. gallinarum-pullorum* (1.7%), *S. newbrunswick* (0.2%) and *S. senftenberg* (2.3%).

Two hundred and forty-eight cultures (7.1%) of E. coli were obtained. Detailed examinations of the isolated strains were carried out by YAMADA. According to him, pathogenic E. coli such as 0–2 (2 strains), 0–26 (4), 0–86 (2) and 0–126 (1) was found among these strains. None of the strains showed hemolytic activity.

TABLE 1. *Isolation of Staphylococci and Other Groups of Organisms from the Yolks of Dead Chick Embryos*

NAMES OF HATCHERIES	NO. OF HATCHES	NO. OF EMBRYOS EXAMINED	ORGANISM			
			Staphylococcus (%)	*Salmonella* (%)	*Escherichia coli* (%)	Enterococcus-like organisms (%)
Tm	3	306	11 (3.6) –2*	57 (18.6)	45 (14.7)	5 (1.6)
Sn	4	250	7 (2.8) –2	8 (3.2)	11 (4.4)	4 (1.6)
Mk	4	292	8 (2.7) –2	8 (2.7)	8 (2.7)	5 (1.7)
Kt	3	259	5 (1.9) –2	7 (2.7)	11 (4.2)	6 (2.3)
Fy	4	216	3 (1.4) –1	26 (12.0)	3 (1.4)	3 (1.4)
Nm	5	246	3 (1.2) –3	14 (5.7)	18 (7.3)	11 (4.5)
Kr	8	715	7 (1.0) –4	14 (2.0)	29 (4.1)	19 (2.7)
Mn	4	855	5 (0.6) –2	18 (2.1)	113 (13.2)	11 (1.3)
Hs	1	36	·	·	·	1 (2.8)
I	2	166	·	2 (1.2)	9 (5.4)	4 (2.4)
Hn	2	122	·	1 (0.8)	1 (0.8)	·
Total	40	3,463	49 (1.4) –18 (45%)	154 (4.4)	248 (7.1)	70 (2.0)

* No. of hatches which yielded embryos infected with staphylococci.

Enterococcus-like organisms were isolated from 70 embryos (2%).

Various kinds of other aerobic bacteria were found frequently, though they yielded mixed or poor cultures.

2. Occurrence of staphylococci in chick down in incubator

Ninety-two samples of chick down collected from 19 hatcheries were examined. Sixty-one samples (66.3%) yielded staphylococcal cultures. Out of them 5 samples from 5 hatcheries gave hemolytic colonies on sheep blood agar; only one of the colonies from the 5 samples was coagulase-positive.

3. Characteristics of staphylococci isolated from embryos and from pathological conditions in chickens

Sixty-three strains including 51 from embryonating eggs, 10 from lesion in young chickens and 2 from secondary infection in fowl pox were examined bacteriologically. The strains of egg origin consisted of 40 from the yolks of embryos, 5 from the egg-white and 6 from the egg-shell.

Biological characteristics of these strains are tabulated in tables 2 and 3. In these tables, strains of each origin are divided into coagulase-positive (33 strains) and coagulase-negative groups (30 strains). Coagulase production of 5 out of 12 strains from lesions and fowl pox infection were weak or labile. These strains include 4 strains from diseased young chickens and one from fowl pox infection. Their coagulases appeared, after overnight incubation, in

TABLE 2. *Coagulase Production of Staphylococci from Dead Chick Embryos, Chicken Lesions and Fowl Pox Infection and their Biological Characteristics*

	ORIGIN OF THE STRAINS				
	Dead embryos (21 strains)	Dead embryos and Egg-shell (30 strains)	Lesions and Fowl Pox (7 strains)	Lesions and Fowl Pox (5 strains)	Cow, Horse and Human Beings (7 strains)
Coagulase production	+	−	+	weak or labile	+
Pigment: yellow	20	13	7	2	6
Pigment: white	1	17	•	3	•
Pigment: lemon yellow	•	•	•	•	1
Nitrate reduction	+	+29	+	+4	+
Gelatin liquefaction	+11	+25	+6	+4	+2
Hemolysis	+20	−	+	+3	+
$NH_4H_2PO_4$ utilization	−	+13	−	−	−
Litmus milk: A. C. P.*	+	+21	+	+4	+
Litmus milk: Alkaline	•	+8	•	•	•
Litmus milk: No change	•	+1	•	+1	•
Methyl-red test	+15	+20	+3	+2	+4
Voges-Proskauer test	+18	+1	+6	+4	+5
Growth on 10% NaCl agar	+	+	+	+	+
Ammonia production	+19	+21	+	+4	+

* A.: Acid production C.: Acid curd P.: Proteolysis

both rabbit and human plasmas or in one of the two. A part of them showed occasional evident production of coagulase.

All except one of the coagulase-positive strains from embryos or from diseased chickens produced yellow pigment, while more than half of the coagulase-negative strains were of white color. It seems to be unusual that coagulase-positive strains frequently did not liquefy gelatin, while the majority of the coagulase-negative strains did liquefy it. In this study stab culture into 30% gelatin media was observed for 2 months. Further experiment is needed since only a few of the controlled pathogenic strains liquefied gelatin.

It may be seen from table 2 that biological characteristics of coagulase-positive strains were considerably uniform. This uniformity was not observed in the groups of coagulase-negative strains or of weak or labile producers of coagulase. The same findings were obtained in fermentation of carbohydrates as shown in table 3. Coagulase-positive strains were active on glucose, sucrose, maltose, lactose, trehalose, mannose, glycerin and mannitol, while they did not ferment arabinose, salicin, raffinose and inulin. Coagulase-negative strains occasionally fermented the last 3 kinds of carbohydrates. On the other hand, none of the coagulase weakly positive strains attacked them.

TABLE 3. *Coagulase Production of Staphylococci of Various Origins and their Carbohydrate Fermentation*

	ORIGIN OF THE STRAINS				
	Dead embryos (21 strains)	Dead embryos and Egg-shell (30 strains)	Lesions and Fowl Pox (7 strains)	Lesions and Fowl Pox (5 strains)	Cow, Horse and Human Beings (7 strains)
Coagulase production	+	−	+	weak or labile	+
Glucose { Aerobic	+	+	+	+	+
Glucose { Anaerobic	+	+	+	+	+
Sucrose	+	+21	+	+	+
Maltose	+	+18	+	+4	+
Lactose	+	+27	+	+4	+
Trehalose	+	+29	+	+	+
Mannose	+	+	+	+	+
Glycerin	+	+28	+	+	+
Mannitol	+	+15	+	+4	+
Arabinose	−	+7	−	−	−
Salicin	−	+14	−	−	+1
Raffinose	−	+6	−	−	−
Inulin	−	−	−	−	−

Recently OCHI and KATSUBE investigated bovine staphylococci; they classified the staphylococci into 3 types on the basis of ability of coagulase production and behavior in fermentation of carbohydrates. In their report, strains which produced coagulase and fermented carbohydrates were called *Staphylococcus* type I, those which did not produce coagulase but fermented carbohydrates *Staphylococcus* type II, and those which did not produce coagulase nor ferment carbohydrates *Staphylococcus* type III. This classification may be applied also to the strains in the present study. Moreover, according to "BERGEY's Manual of Determinative Bacteriology" (1957), the coagulase-positive strains examined belong to *Staphylococcus aureus* and the coagulase-negative and non-mannitol-fermenting strains to *Staphylococcus epidermidis*. However, it is an interesting fact that in the present study half of the coagulase-negative strains fermented mannitol.

In general, production of coagulase and of hemolysin is regarded as an important characteristic of pathogenic staphylococci. However SMITH[17] stated that inability to produce hemolysin is a characteristic of many poultry pathogenic strains. Table 4 indicates the ability of hemolysin production in the strains of each origin. As shown in this table, more than 40 per cent of the embryonic strains produced both coagulase and hemolysin. Out of 33 coagulase-positive strains isolated from dead chick embryos and from chicken lesions, only 3 strains were non-hemolytic or weakly so. In the majority of the hemolytic strains, δ-hemolysin was produced.

TABLE 4. *Relationship between Origin of the Staphylococcal Strains and their Abilities of Coagulase and Hemolysin Production*

ORIGIN		LOCALITY	NO. OF STRAINS	C+ H+	C+ H−	C− H−	HEMOLYSIN TYPE α	δ	αδ	βδ
Embryonating eggs	Yolk	8 hatcheries	40	17 (42.5%)	1	22	·	8	·	9
	Egg-white	2 "	5	3	·	2	·	3	·	·
	Egg-shell	1 hatchery	6	·	·	6	·	·	·	·
Chiken lesions		Gifu	5	5	·	·	1	1	1	2
		Sapporo	2	1	1	·	·	1	·	·
		Italy	3	3	·	·	·	2	·	1
Secondary infection in fowl pox		2 districts	2	1	1	·	·	1	·	·
Total			63	30 (47.6%)	3	30	1	16	1	12

C: Coagulase H: Hemolysis

4. Occurrence of coagulase-positive staphylococci in hatcheries

Eighteen of 40 strains which were isolated from the embryonic yolk were coagulase-positive; they were derived from 4 out of 11 hatcheries and from 4 of a total of 40 hatches. This may suggest that the occurrence of coagulase-positive staphylococcus is uncommon in incubator.

5. Pathogenicity of the isolated strains

Broth cultures of 13 strains, 4 of which were coagulase-negative, were centrifuged and the sediments were resuspended in broth so as to contain about 800 million of the organisms in 0.5 ml. Three mice, one rabbit and 1~2 young White Rock chickens were inoculated with the above-described dose of each strain.

Rabbits and mice died within 4 days and chickens 4~12 days after inoculation. Toxemia from large dose of inocula might be responsible for the death of the rabbits and the mice. On the other hand, in the chickens, clinical signs were observed before death as described by SMITH[18].

As shown in table 5, six strains of coagulase-positive staphylococci of different origins killed the animals, especially chickens without exception. However, 3 strains which originated from pathological lesions in chickens and showed weak or labile production of coagulase, did not kill chickens. Four coagulase-negative strains did not reveal pathogenicity at all. From these results, it will be seen that the coagulase test is most suitable in determination of pathogenicity of staphylococci as pointed out by previous workers, but the strength of coagulase production should be considered in the determination. Anyhow it is of interest that the strains showing weak or labile coagulase production are isolated from pathological conditions in chickens. Staphylococci with this character may be essentially secondary invaders in chicken diseases.

TABLE 5. *Pathogenicity of Staphylococcal Strains of each Origin in Relation to their Coagulase and Hemolysin Production*

ORIGIN	NO. OF STRAIN	COAGULASE	HEMOLYSIN TYPE	MICE (about 20 g) ip	RABBITS (about 1500 g) iv	CHICKENS (about 600 g) iv
Embryos	2	+	βδ	3/3*	1/1*	1/1*
	12	+	δ	3/3	0/1	2/2
	52	+	±	1/3	1/1	2/2
	6	−	−	1/3	0/1	0/2
	31	−	−	0/3	0/1	0/1
	37	−	−	0/3	•	0/1
	43	−	−	0/3	0/1	0/1
Lesions in young chickens	56	+	αδ	3/3	1/1	1/1
	61	+	βδ	3/3	1/1	1/1
	58	weak	βδ	3/3	1/1	0/1
	59	labile	α	0/3	0/1	0/1
	66	weak	—	0/3	0/1	0/2
Secondary infection in fowl pox	67	+	δ	3/3	1/1	1/1

* Denominator: No. of animals inoculated
Numerator : No. of deaths

6. Slide-agglutination test by means of unabsorbed sera

In order to ascertain any antigenic relation between the strains derived from embryos and those from chicken lesions, or among the other strains examined, slide-agglutination test was made using unabsorbed sera prepared from seven strains including 4 from embryos and from lesions. These strains had been derived from 4 hatcheries and 3 districts respectively. Twenty-one coagulase-positive strains from embryos and 10 coagulase-positive strains from chicken lesions were tested. Nine pathogenic strains of human origin which were forwarded from Dr. OEDING to Dr. TAJIMA were employed as the control.

Each serum diluted 1 : 10 agglutinated 30 strains out of 31 and the controlled human strains. The non-agglutinated strain was coagulase weakly positive and showed atypical characteristics, such as no reduction of nitrate, no change of litmus milk and no fermentation of lactose.

A tentative investigation was made to ascertain the agglutinability of the strains employed. Each serum was diluted in the highest dilution such as 1 : 500～1 : 1,000, which yielded agglutination of homologous strains visible to the naked eye. One of 9 human strains was agglutinated by the above-mentioned 7 sera, 3 of the strains were agglutinated by 1 or 2 sera. The remaining 5 strains did not react to any of the sera. One strain of chicken lesion was agglutinated by 2 of 3 sera of strains from chicken lesion but not by 4 sera prepared from embryonic strains. The antiserum of this strain developed no agglutination of any of the strains of embryonic origin. On the other hand, except one strain which was not agglutinated by any of the sera in dilution 1 :10, eight of the remaining 9 strains from chicken lesion were

agglutinated by this serum.

The above-noted inagglutination in the highest dilution of the sera may show either quantitative or qualitative difference in antigens between the homologous strains and the non-agglutinable ones. This seems to indicate some serological difference between chicken strains and those of human origin, and among chicken strains. However, detailed investigation on serological typing of embryonic strains or of those from diseased chickens should be made using absorbed factor serum.

7. Investigation on bacteriophage typing

Seventy-one strains from dead chick embryos, pathological conditions in chickens, and chick down were examined on the basis of the phages of the International Series or of NAKAGAWA's[13] Bovine phages.

TABLE 6. *Susceptibility of the Isolated Staphylococci to the Phages of the International Series*

ORIGIN	NO. OF STRAINS	COAGULASE PRODUCTION	TYPABLE				UNTYPABLE			
			I	III	II+III	Total	II	III	Mixed*	Total
Embryos	21	+	·	3	·	3	6	2	5	13
	30	−	·	·	·	·	4	·	1	5
Chicken lesions	6	+	1	1	·	2	1	·	1	2
	4	weak or labile	·	·	·	·	1	·	·	1
Secondary infection in fowl pox	1	+	·	·	·	·	1	·	·	1
	1	labile	·	·	·	·	·	·	·	·
Chick down	1	+	·	·	·	·	1	·	·	1
	7	−	·	·	1	1	3	·	·	3
Total	71		1	4	1	6	17	2	7	26

* Mixed types include I+II+III, I+II, II+III and II+IV.

As indicated in table 6, six out of the 71 strains (5 of 34 coagulase-positive strains and 1 of 37 coagulase-negative ones) were typable (8.5%) by the International phages. Three of the typable strains were also lysed strongly by phages at 1×R.T.D. Only 4 strains including one Italian strain were lysed by phage 53 and were typed into Type III. It is interesting that 3 of 4 strains typed into Type III were traceable to the same hatch of one hatchery.

Twenty-six of 65 untypable strains of coagulase-positive or negative staphylococci were susceptible to one or more of the phages; specifically, they were lysed weakly by phages at 10×R.T.D. or 100×R.T.D. and sometimes at 1×R.T.D. Most of the strains were susceptible to Group II phages, especially to phage 71. Nine strains out of 22 susceptible to phage 71 were coagulase-negative. Seven strains, 6 coagulase-positive and 1 negative, were susceptible to more than 2 phages of the same group, and 5 of them were typable.

On the other hand, only 2 of 71 strains were lysed slightly by Bovine phages.

From the above-noted facts, no significant correlation could be found between phage type

and origin or locality of the strains, though 3 of 8 strains isolated from embryos of one certain hatchery were typed into Type III.

Lysogenicity was proven in only one coagulase-negative strain which was slightly susceptible to the phages of Groups II and III of the International Series.

Phage isolated from this lysogenic strain was active on 26 of 29 coagulase-positive strains from embryonating eggs and chicken lesions. Out of 3 coagulase-positive non-susceptible strains, 2 were weak or labile in production of coagulase. None of the strains of coagulase-negative staphylococci was susceptible to the phage, but 2 coagulase-positive strains of horse origin were lysed by it. It is an interesting fact that a majority of the coagulase-positive chicken strains were susceptible to the phage. This may show an uniformity in the characteristics of coagulase-positive staphylococci of chicken origin.

Discussion

In the present study, frequency of isolation of staphylococci from dead chick embryos was the lowest compared with that of *Salmonella*, *E. coli* and enterococcus-like organisms. This may be due to the technique employed for bacteriological examination. That is, a part of staphylococcal growths might not have been noted, since the authors employed the strains that originated from dominant or almost pure cultures of micrococcus-like colonies.

Itagaki and Tsubokura carried out bacteriological investigations on dead chick embryos and on newly hatched chicks which were in poor health conditions or which died soon after hatching. They most frequently isolated *Micrococcaceae* from dead chicks as well as from dead embryos. Staphylococcal cultures were obtained from 74 (about 7%) out of 1,062 embryos. Moreover they stated that 17 (about 23%) of 74 staphylococcal cultures from embryos and one of 139 from chicks produced coagulase and hemolysin.

More than 40 per cent of the present authors' embryonic strains had characteristics of *Staphylococcus aureus*. Most of the remaining strains isolated from yolks liquefied gelatin. Accordingly, these strains showing proteolytic activity may be pathogenic for embryonic yolk as described by Harry.

No marked difference of biological characteristics was observed between the coagulase-positive staphylococci of dead embryos and those of chicken lesions. Strains of both origins indicated uniformity of biological characteristics and pathogenicity. However, some strains from chicken lesions, weak or labile coagulase producers, showed somewhat variable biological characteristics and could not kill young chickens. Although they were isolated from gangrene on the wing or from the liver of chickens died from septicemia, they might be pathogenic only under lowered resistance of the host.

Slide-agglutination test by means of unabsorbed sera appeared to be little useful in differentiating antigens of the isolated staphylococci, though the test did

differentiate the antigens of one strain with atypical characteristics from those of the other strains examined. Therefore, staphylococci of chicken origin may have considerably similar antigens as SMITH[17] reported stating that most of the strains tested by him belonged to the same serological type. Moreover, TORLONE (1957) stated that 75% of the chicken staphylococcal strains in his study were of the same serological type.

SMITH[17] reported that 8 of 13 strains of chicken origin were susceptible to the phages of the Staphylococcal Reference Laboratory, Colindale. HARRY reported that *Staphylococcus aureus* which caused yolk infection both in embryos and in chicks was considered to be of human origin on account of its phage type. The organism was traced to the persons involoved in handling the eggs on the 18th day, who were found to be either nasal or skin carriers of this organism. In Italy, TORLONE (1958) reported that 22 of 35 avian strains were lysed by phage 47C. In addition, MONDINI and DOVADOLA described 29 strains of 76 of staphylococci from fowls as susceptible to the same phage. In the present study, this phage was not employed. Therefore, it is impossible to determine the difference of phage susceptibility between Italian and Japanese strains. However, one of 3 chicken strains submitted from Italy was strongly lysed by phage 53 and the remaining 2 were weakly susceptible to phage 71. So far as these 3 Italian strains are concerned, their phage susceptibility seems to suggest a similar tendency to that in the strains isolated by the present authors. MINER et al.[7] reported that most of staphylococcal strains isolated from turkey staphylococcosis belonged to International phage Type III. Moreover, MINER and SMART[8] stated that in respect to phage type there seems to be an intimate relation between the staphylococci of human origin and those of turkey. Recently SMITH and CRABB reported that, on the basis of phage typing and other tests, the antibiotic resistant strains isolated from the attendants were usually identical with those isolated from their animals including chickens and pigs. As indicated by the above-mentioned workers, phage typing is considered to be of value in the epidemiological study of avian staphylococcal infections.

In the present study, 3 out of 8 coagulase-positive strains isolated from the same hatch of a certain hatchery were typed into the same type. This may indicate an outbreak of staphylococcal infection among embryonating eggs in the hatch. It should be emphasized that new typing phages isolated from avian staphylococci are required for the epidemiological study of avian staphylococcal infections because of the fact that only a few strains of staphylococci tested were typable by both the International phages and the Bovine phages.

SMITH[19] stated that he isolated phages being active only on *Staphylococcus aureus* of poultry origin. The present authors isolated a phage from a lysogenic

strain. The phage was active on most of the strains of coagulase-positive staphylococci of chicken origin and also on 2 horse strains.

SUMMARY

Some investigations were carried out on the staphylococci isolated from dead-in-shell chick embryos and from diseased chickens. The results obtained are summarized as follows:

1. Staphylococci were isolated from the yolks of 49 (1.4%) out of 3,463 dead embryos collected from 11 hatcheries.
2. Eighteen (45%) of the isolated 40 strains were coagulase-positive.
3. One of 92 samples of chick down in incubator collected from 19 hatcheries yielded coagulase-positive staphylococci.
4. Coagulase-positive strains from dead chick embryos and from chicken lesions indicated characteristics of *Staphylococcus aureus* (BERGEY's Manual, 1957) and were pathogenic for chickens.
5. A part of the strains from chicken lesions were weak or labile in coagulase production, and they appeared to be low or non-pathogenic. Some of them showed atypical biological characteristics. In addition, one of them differed in serological character from coagulase-positive strains from chicken lesion or embryos.
6. Thirty-two (45.1%) of 71 strains (34 coagulase-positive and 37 negative) which were isolated from dead chick embryos, chicken lesions, secondary infection in fowl pox and chick down were susceptible to the phages of the International Series. Six of the susceptible strains were typable as follows; Type I (1 strain), Type III (4) and Type II+III (one coagulase-negative strain from chick down).

Lysogenicity was proven in a coagulase-negative strain from embryo. The phage of this strain was slightly active on most of the strains of coagulase-positive staphylococci of chicken origin.

7. From the results obtained from examination of dead embryos and of samples of chick down, *Staphylococcus aureus* seems to be inclined to occur only in a part of the hatches of some hatcheries.

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