THE SEROLOGICAL DIFFERENTIATION OF PATHOGENIC AND NON-PATHOGENIC STRAINS OF HEMOLYTIC STREPTOCOCCI FROM PARTURIENT WOMEN*

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Hemolytic streptococci may cause several different clinical types of infection or, on the other hand, may be merely saprophytic and harmless to the human host. Acute tonsillitis and scarlatina, for example, are due to hemolytic streptococci, but a proportion of normal individuals habitually carry hemolytic streptococci in the throat without any apparent harm, and so far as it is known, without infecting other people. The organisms from both infected throats and healthy carriers are indistinguishable by any known test, and why one group of persons should be sick and the other go unscathed has never been satisfactorily explained.

The same phenomenon occurs in the human birth canal before and after delivery; most severe infections of the uterus which follow childbirth are due to hemolytic streptococci (1, 2); but these organisms may be present in the birth canal before and after delivery without the host showing any sign associated with active infection (3, 4).

Taylor and Wright (3) were unable to distinguish, by any of the tests at their disposal, between the organisms isolated from the vagina of febrile and afebrile cases. Hare (5) has, however, advanced evidence showing that most of the saprophytic[1] strains can be differentiated from the infecting strains in being sus-

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[1] The terms "saprophytic" and "non-infective" are used throughout this paper to imply failure to induce disease in the human host, although it is known that some, at least, of the strains which are saprophytic in the human vagina are nevertheless highly pathogenic for certain other animals.

ceptible to the bactericidal action of normal human blood.   Hare and Colebrook (6) have also shown that a large proportion of the saprophytic strains in the vagina can be differentiated from infecting strains, since many of the former are, apparently, members of the group of hemolytic streptococci which causes a large proportion of cases of bovine mastitis.   These workers employed biochemical tests to differentiate their strains; but Lancefield (7) has shown that it is possible to distinguish mastitis and other animal-infecting strains from strains from human infections by precipitin tests.   She described four groups, each characterized by its own precipitinogen, or C substance.   Group A consisted almost entirely of organisms from human infections (these strains giving a pH of 5.0 to 5.4 in 1 per cent glucose broth and failing to hydrolyze sodium hippurate); Group B, of organisms mainly from bovine mastitis (hydrolyzing sodium hippurate and giving a low pH in 1 per cent glucose broth); Group C, of organisms from a variety of animal sources (failing to hydrolyze sodium hippurate, giving an intermediate pH in 1 per cent glucose broth, and usually differing from the former groups in fermenting sorbitol and not trehalose); and Group D, a group of eight strains derived from cheese (giving a low pH in 1 per cent glucose broth, having little or no action on sodium hippurate, fermenting both sorbitol and trehalose, and reducing methylene blue milk).[2]

As the majority of the saprophytic strains isolated by Hare and Colebrook (6) gave the biochemical reactions of members of one or other of the serological Groups B and D described by Lancefield, the whole series of their strains were re-examined in order to determine whether:—(a) the strains which they had differentiated by biochemical reactions could also be differentiated by a serological test (precipitin reaction); (b) certain strains from uninfected cases which could not be differentiated biochemically from infecting strains were or were not members of Lancefield's Group A when tested serologically. The following experiments were therefore designed in order to elucidate these points.

Source of Strains

Series 1.   From Cases of Definite Puerperal Uterine Infection.—These strains, 46 in number, were obtained from clinically typical cases of puerperal infection, many of which were fatal, in the Isolation Block of Queen Charlotte's Hospital, London.   These women came from all parts of London after infection had developed.   Only one of them had been delivered in Queen Charlotte's Maternity Hospital, and none came from the District.

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[2] Lancefield also described a fifth Group E, of which she had but three representatives.

Series 2. From Women after Delivery Who Were either Uninfected or Who Had Only a Slight Increase of Temperature during the Puerperium.—Vaginal swabs were taken on the 3rd or 4th day after delivery from the majority of women delivered in Queen Charlotte's Maternity Hospital, over a period of 9 months. Cultures were made irrespective of whether the patient had fever or other signs of an abnormal puerperium. Streptococci giving areas of hemolysis on blood agar plates were isolated from 85 of the 837 women so examined. One of the 85 women was definitely infected, and she died 8 days after delivery from peritonitis and septicemia. The strain from this woman is included in the preceding series. 65 of the women had no elevation of temperature during the puerperium (a temperature of 100°F. on only one occasion being adjudged sufficient evidence to exclude a woman from this category) and it was therefore assumed that despite the presence of the organisms in the birth canal, no active infection was present. The remaining 18 women in this series had some fever during the puerperium but not enough to warrant their definite diagnosis as cases of puerperal uterine infection. Moreover, some of the women had extragenital infections of sufficient severity to cause the elevation of temperature which was observed.

Series 3. From Pregnant Women Immediately before Delivery.—Swabs were taken from the upper part of the vagina of every woman on the District of Queen Charlotte's Hospital immediately before delivery and before examinations of any sort had been made. In thirteen of the 855 women so examined, hemolytic streptococci were found to be present.[3] Twelve of these women with positive cultures subsequently had an afebrile puerperium, although hemolytic streptococci were still present after delivery in the majority of them. The other carrier had some fever during the puerperium, but it was slight and there were no other signs of puerperal uterine infection. Of the remaining 842 women, none subsequently became infected with hemolytic streptococci. For these strains we are indebted to Dr. R. M. Fry.

Isolation of the Strains

The original cultures were made on horse blood agar plates and incubated anaerobically in a McIntosh and Fildes jar for 16 hours (6, 8). Any hemolytic colonies were fished and the cultures purified by plating. The strains were stored in Robertson's meat medium; and subcultures in 5 per cent horse serum broth were inoculated into the various media employed for the tests.

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[3] Only organisms able to form soluble hemolysin were studied from this series of patients. For this reason pseudohemolytic streptococci (strains giving marked hemolysis on blood plates but unable to form soluble hemolysin in the routine tests employed, *vide infra*) although known to be present, were not collected and studied.

Methods

1. Immune Sera.—Immune sera were prepared by the injection of rabbits with formalinized cultures as previously described (7).

2. Extracts.—Extracts were made by heating the organisms with hydrochloric acid as in the earlier experiments (7).

3. Precipitin Tests.—Most of the serological tests were made in America by one of us, according to methods already fully described, with strains sent from England. The remaining tests were carried out in England by the other author with sera sent from America; in the latter tests capillary pipettes as used by Day (9) were employed with the same relative proportions of serum and extracts as were used by Lancefield but in much smaller volume. The actual technique was as follows:

Two volumes (about 5 c.mm. per volume) of undiluted extract or extract diluted one-fourth with saline, were mixed with one volume of undiluted serum on a paraffined slide. The mixture was aspirated into a capillary pipette, which was then sealed, incubated at 37°C. for 2 hours, and placed in the ice chest overnight. The precipitates were easily visible with a hand lens. Control tests with larger volumes of serum and extract were found to give comparable results.

4. Biochemical Reactions.—These consisted of the following tests which were performed and described previously by Hare and Colebrook (6):—(a) formation of soluble hemolysin; (b) final pH after growth for 4 days in 1 per cent glucose broth; (c) hydrolysis of sodium hippurate; (d) growth on 40 per cent bile blood agar; (e) digestion of human fibrin; (f) fermentation of sorbitol, trehalose, lactose, mannite, and salicin.

RESULTS

Series 1. Strains from Definite Puerperal Infections of the Uterus. —In Table I are given the serological and biochemical tests carried out with 46 strains from definite cases of puerperal uterine infection. All except one strain were classified in Group A as a result of the precipitin tests, which showed a sharp and unmistakable differentiation of the various groups. The biochemical reactions of these hemolytic streptococci were, in the majority of instances, of the type usually associated with strains from human infections although some exceptional strains were encountered. It is also noteworthy that all the Group A strains were able to digest human fibrin (10, 11). The one exception to the serological similarity among these strains from infected cases was a Group G strain isolated from the blood of a fatal case of septicemia. The patient was also suffering from an overwhelming infection with *Staphylococcus aureus*, and it is possible that

the hemolytic streptococci were secondary invaders. It was also noted that biochemically this strain was very atypical.

Series 2. Strains Isolated after Delivery from Women Who Were either Uninfected or Who Had Only a Slight Increase of Temperature during the Puerperium.—The serological and biochemical reactions of the 66 strains isolated on the 3rd or 4th day of the puerperium from the 65 women who had no elevation of temperature at any time during the puerperium (members of two groups being isolated from one patient) are given in Table II.

TABLE I

Series 1: Strains from Severe Cases of Hemolytic Streptococcal Infection of the Uterus

No. of strains	Precipitation with sera from Group						Formation of soluble hemolysin	pH in 1 per cent glucose broth	Hydrolysis of sodium hippurate	Growth on 40 per cent bile agar	Digestion of human fibrin	Fermentation of				
	A	B	C	D	F	G						Sorbitol	Trehalose	Lactose	Mannite	Salicin
37	+	−	−	−	−	−	++++	5.0–5.4	−	−	+	−	+	+	−	+
1	+	−	−	−	−	−	++++	4.9	−	−	+	−	+	+	−	+
3	+	−	−	−	−	−	++++	5.2–5.4	−	−	+	+	+	+	+	+
1	+	−	−	−	−	−	++++	5.0	−	+	+	+	+	+	+	+
1	+	−	−	−	−	−	++++	5.0	−	±	+	−	+	+	−	+
1	+	−	−	−	−	−	++++	5.4	−	−	+	−	+	±	+	+
1	+	−	−	−	−	−	++++	5.4	−	−	+	−	−	+	−	+
1	−	−	−	−	−	+*	+	5.0	−	+	−	−	+	+	−	+
Totals...46	45	0	0	0	0	1										

* Patient had *Staphylococcus aureus* septicemia.

Only one Group A strain was isolated from these afebrile patients, and this was the only instance in this study in which a Group A strain was found in the vagina unassociated with disease. All of the other strains from afebrile patients post partum, fell into other groups or were unclassified; the 26 members of Group B were, with one exception, also distinguishable from other groups by their biochemical reactions, as were also the 26 members of Group D. The five members of Group C, as well as the two Group F, the three Group G, and the three unclassified strains, were somewhat variable in their biochemical reactions but could be clearly differentiated from the pathogenic strains by means of the precipitin test.

In Table III are given the reactions of the 18 strains from the 18 women in this series who had some degree of fever during the puerperium. It must be stressed that there was much doubt with most of these women whether this pyrexia was really due to a uterine infection. Certainly none of the cases could be compared with those enumerated in Table I in point of severity. The relevant clinical

TABLE II

Series 2: Strains Isolated Post Partum from Women with an Afebrile Puerperium

No. of strains	Precipitation with sera from Group						Formation of soluble hemolysin	pH in 1 per cent glucose broth	Hydrolysis of sodium hippurate	Growth on 40 per cent bile agar	Digestion of human fibrin	Fermentation of				
	A	B	C	D	F	G						Sorbitol	Trehalose	Lactose	Mannite	Salicin
1	+	−	−	−	−	−	++++	5.4	−	−	+	−	+	+	−	+
25	−	+	−	−	−	−	+ to +++	4.2–4.9	+	+	−	−	+	+	−	+
1	−	+	−	−	−	−	+	4.6	−	+	−	+	+	−	−	−
2	−	−	+	−	−	−	++++	5.4	−	−	+	−	+	+	−	+
1	−	−	+	−	−	−	++++	5.0	−	+	+	−	+	+	−	+
1	−	−	+	−	−	−	++++	5.4	−	−	+	−	+	+	−	−
1	−	−	+	−	−	−	++++	5.2	−	−	+	−	+	+	−	−
26	−	−	−	+	−	−	−	4.2–4.8	−	+	−	+	+	+	+	+
1	−	−	−	−	+	−	+	5.0	−	−	−	−	−	+	−	+
1	−	−	−	−	+	−	+	5.0	−	−	−	−	+	+	−	+
3	−	−	−	−	−	+	++++	5.2–5.4	−	−	+	−	+	+	−	+
1*	−	−	−	−	−	−	++++	5.4	−	−	−	−	+	+	−	+
1*	−	−	−	−	−	−	+++	4.8	−	−	−	−	+	+	−	+
1	−	−	−	−	−	−	−	4.8	−	+	−	+	+	+	+	+
Totals..66†	1	26	5	26	2	3										

* These two strains was also tested with sera of Group E and Strain K 130. Negative reactions were obtained.

† 3 unclassified strains are included in this total.

details of these "minor infections" are also given in Table III. It is evident that none of the 18 strains fell into Group A. One strain was in Group C, one in Group G, and one unclassified; seven strains were members of the bovine mastitis Group B, and eight were "pseudo-hemolytic streptococci," members of Group D. It would seem, therefore, that some of the minor infections which accompany childbirth may possibly be due to organisms of other groups than Group A.

TABLE III

Series 2: Strains Isolated from Minor Infections

Name	Temperature	Serological group	Formation of soluble hemolysin	pH in 1 per cent glucose broth	Hydrolysis of sodium hippurate	Growth on 40 per cent bile agar	Digestion of human fibrin	Fermentation of				
								Sorbitol	Trehalose	Lactose	Mannite	Salicin
W-y	101°F. on 2nd day	C	++++	5.0	−	−	+	−	+	+	−	+
M-d	100.6°F. on 4th and 5th days	G	++++	5.2	−	−	+	−	+	+	−	+
B-y	99.4°F. on 3rd, 100.4° on 4th, 99.4° on 5th days	B	++	4.4	+	+	−	−	+	+	−	+
S-s	100°F. on 2nd, 4th, and 6th days	B	+++	4.6	+	+	−	−	+	+	−	+
T-n	101.4°F. on 5th, 100.2° on 6th days	B	+++	4.5	+	+	−	−	+	+	−	+
M-n	99–100°F. for 7 days	B	+	4.7	+	+	−	−	+	+	−	+
C-e	103°F. on 4th day (cold ?)	B	+	4.6	+	+	−	−	+	+	−	+
C-k	100°F. on 1st day	B	+	4.5	+	+	−	−	+	+	−	+
W-f	100°F. on 2nd, 101.4° on 6th, and 102.8° on 8th days (influenza ?)	B	+	4.8	+	+	−	−	+	+	−	+
P-e	101.4°F. on 7th, 101.8° on 8th days (probably due to breast abscess)	D	±	4.6	±	+	−	+	+	+	+	+
S-d	102°F. on 1st day	D	−	4.5	−	+	−	+	+	+	+	+
B-m	101.6°F. on 8th day	D	−	4.5	±	+	−	+	+	+	+	+
M-n	102°F. on 1st day	D	−	4.5	±	+	−	+	+	+	+	+
C-r	101.6°F. on 2nd, 100.4° on 3rd, 99.4° on 5th days	D	−	4.7	±	+	−	+	+	+	+	+
F-g	100°F. ± for 7 days	D	−	4.6	−	+	−	+	+	+	+	+
B-t	100–102°F. for 7 days	D	−	4.6	−	+	−	+	+	+	+	+
D-g	100°F. on 5th, 100.4° on 8th days (pyelitis)	D	−	4.5	−	+	−	+	+	+	+	+
L-n	100–101°F. for 4 days	U*	+++	5.4	−	−	+	−	+	+	−	+

* Indicates unclassified strain.

Thus, in the whole series of 837 women examined after delivery in Queen Charlotte's Maternity Hospital, organisms giving areas of hemolysis on blood agar were isolated from (a) one undoubted case of puerperal infection, (b) 18 cases of minor infection, and (c) 65 women who had an absolutely afebrile puerperium. The strain from the definitely infected case fell into Group A, and, with the exception of one Group A strain from an afebrile patient, none of the strains from the afebrile patients or those with minor infection were identified as members of Group A.

TABLE IV

*Series 3: Strains Isolated Ante Partum from Women Whose Subsequent Puerperium Was Afebrile**

No. of strains	Precipitation with sera from Group						Formation of soluble hemolysin	pH in 1 per cent glucose broth	Hydrolysis of sodium hippurate	Growth on 40 per cent bile agar	Digestion of human fibrin	Fermentation of				
	A	B	C	D	F	G						Sorbitol	Trehalose	Lactose	Mannite	Salicin
5	−	+	−	−	−	−	+ to ++	4.6–4.8	+	+	−	−	+	+	−	+
1	−	−	+	−	−	−	++++	5.4	−	−	+	−	+	+	−	+
3	−	−	−	−	−	+	++++	5.2–5.4	−	−	+	−	+	+	−	+
2	−	−	−	−	−	+	++	4.6	−	+	−	−	+	+	−	+
Total....11	0	5	1	0	0	5										

* Group B strains were isolated both before and after delivery from one additional patient who had a minor degree of pyrexia during the puerperium.

Series 3. Strains from Cultures Taken Immediately before Delivery. —The reactions of eleven of the twelve strains (one strain was lost) isolated before delivery from women whose subsequent puerperium was afebrile are given in Table IV. It will be seen that none of these strains, saprophytic in the vagina, fell into Group A. The remaining patient had slight pyrexia during the puerperium and the strains isolated before and after delivery were both found to belong to Group B by serological and biochemical tests.

It is thus clear that the vast majority of strains from severe puerperal infections due to hemolytic streptococci are members of Group

A, and that most of those which are saprophytic in the birth canal either before or after delivery, as well as those strains isolated from women with minor degrees of fever, do not belong to Group A. Hare and Colebrook (6) were able to differentiate by means of biochemical tests the majority of these saprophytic strains (those placed in Groups B and D in this paper) from the strains isolated from infections. The present work confirms their findings in this respect. However, the serological tests reported here show that some non-infective strains which Hare and Colebrook found biochemically similar to those from infections but which they were unable to differentiate from infecting strains can, nevertheless, be differentiated by their group precipitinogen (those placed in Groups C, F, and G in this paper). The information available on these points may be summarized in the form of a table (Table V).

Comments on the Serological and Biochemical Reactions of Strains from the Different Groups

Facts relevant to the serological groups into which these organisms have been classified, and a description of the two new serological groups found in this series of hemolytic streptococci are given below:—

Group A.—The Group A strains, derived from human puerperal infections of a severe type, all form a C substance which gives precipitates with the appropriate Group A anti-C sera. Their biochemical reactions, with a few exceptions, are those of *Streptococcus pyogenes.*

Group B.—Organisms placed in this group, by reason of their serological reactions, give the biochemical reactions of certain bovine mastitis organisms; that is to say, they hydrolyze sodium hippurate, give a low pH in 1 per cent glucose broth, grow on 40 per cent bile agar, and form only small amounts of soluble hemolysin.

The Group B strains in the present series of hemolytic streptococci isolated from women were classified into types in order to determine whether they belonged to the same serological types encountered among the Group B strains derived from cows (12). The majority of the strains (34 out of 39) were tested and all except one strain fell into one or other of the specific types originally differentiated among strains of bovine origin. In all, 39 strains of this group have been isolated from the human vagina before or after delivery. Plummer (13) has also found organisms of this group in normal human throats. One of us has confirmed this. There can be little doubt, therefore, that Group B organisms can

exist as saprophytes in the human throat and vagina. But in view of the evidence set forth in Table III it would seem that a proportion of low grade infections may be due to organisms of this group.

Group C.—Group C (7) contains strains which are similar biochemically to pathogenic human strains but which are distinguishable by the specific anti-C

TABLE V

Serological Grouping of All Strains Examined

Time of culture	No. of women examined	Clinical condition of patient	No. of strains isolated	No. of strains in each serological group						
				A	B	C	D	F	G	Unclassified
Before delivery (Queen Charlotte's Hospital District)	855	Afebrile puerperium Minor infection	11* 1		5 1	1	†		5	
After delivery (Queen Charlotte's Hospital)	837	Afebrile puerperium Minor infection Definite infection	66‡ 18 1	1 1	26 7	5 1	26 8	2	3 1	3 1
After delivery (Queen Charlotte's Hospital Isolation Block)§	45	Definite puerperal infection	45	44					1‖	
Totals			142	46	39	7	34	2	10	4

* One more strain was isolated but was lost.

† Only organisms which formed soluble hemolysin were studied in this group of patients. All pseudohemolytic streptococci studied from other patients have fallen into Group D.

‡ Members of two groups (B and G) were isolated from one patient.

§ The members of this group were not derived from the groups studied in Queen Charlotte's Hospital and on the District. These patients were brought to the Isolation Block, which is entirely separate from the Maternity Hospital proper, from various parts of London at a time when they were already ill.

‖ Also accompanying overwhelming *Staphylococcus aureus* infection.

reaction. The strains originally studied were all derived from animal sources other than man, gave marked hemolysis on blood agar plates, and formed soluble hemolysin. They usually differed from *S. pyogenes* of human origin in fermenting sorbitol and not trehalose, although four strains among the 49 examined by Lancefield (7) fermented neither of these substances. Other points of difference were in the attainment of a final pH in 1 per cent glucose broth inter-

mediate between that of Group A strains and that of Group B strains, and in the susceptibility of Group C strains to lysis by bacteriophage. Edwards (14) and also Ogura (15) have described hemolytic streptococci having similar characteristics, all derived from animals.[4] Edwards has also shown that the strains he studied can be differentiated from human strains by precipitin tests, with the carbohydrate specific for Group C. In the present study, seven strains have been encountered, giving group precipitates with Group C antisera. All except one were saprophytic, and there is much doubt whether the remaining strain was really infecting the host. All these seven strains fermented trehalose and not sorbitol. In this respect they differed from the Group C strains from animal sources described by Lancefield, but were similar to the Group C strains recently described by Edwards and designated by him in conformity with Ogura's nomenclature, as Types B 1 and B 2. The seven Group C strains described in the present paper all ferment lactose in addition to trehalose, and would on this basis belong to Edwards' Type B 2, of which he described one strain and Ogura three. All seven were found capable of digesting human fibrin.

It may be mentioned in passing that one of us has also obtained from animal sources Group C strains which ferment trehalose but not sorbitol or lactose. One was from the throat of a normal dog and three were from the throats of *cynomolgos* monkeys. The other author has encountered sixteen Group C strains in the throats of normal human beings all of which fermented trehalose and lactose but not sorbitol.

It is of interest that the fermentation of salicin is somewhat variable among strains placed in this group. A definite proportion of the strains fail to ferment this substance.

Group D.—The eight strains of this group described by Lancefield (7) were all derived from cheese. Of the present series of strains, 34 were found to belong to this group, and their biochemical reactions were in all important respects similar to those originally studied by Lancefield. These 34 strains were described by Hare and Colebrook (6) as "pseudohemolytic streptococci" because they differed from organisms placed in the other groups in not forming soluble hemolysin, when tested by the usual methods,[5] although giving complete and wide areas of hemolysis on blood agar plates. They also regularly ferment mannite and sorbitol as well as trehalose, lactose and salicin, and give a low pH in 1 per cent

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[4] It is probable that the S 21 series of Minett and Stableforth (16) are similar organisms.

[5] The actual method consisted in the addition of one volume of a 12 to 16 hour culture in 20 per cent horse serum broth to one volume of 5 per cent horse red cells in saline and incubating the mixture at 37°C. for 2 hours. Todd (17) has since shown that by employing a highly buffered broth and a relatively lower temperature (30°C.) for the incubation of the cultures, Group D organisms do form sufficient soluble hemolysin to give a positive test when the cultures are added to red cells in saline.

glucose broth but have little or no action on sodium hippurate. Similar organisms have been described by Taylor and Wright| (3) as occurring in the vagina, and by Weatherall and Dible (18) in the feces. One of us has confirmed this latter finding. These pseudohemolytic streptococci resemble the group of entero-cocci or *S. faecalis* more than *S. pyogenes* because they are characteristically lanceolate, grow in moist luxuriant colonies, and a proportion resist heat at 60°C. for 30 minutes. It is improbable that organisms of this group are responsible for severe infections, although one case of endocarditis due to them has been encountered.[6]

Group F.—This is a new group not hitherto described. The original members (four strains) were obtained from Dr. Perrin H. Long. Their morphology was originally described in abstract by Bliss and Long (20) and in more detail recently (21). Their chief characteristics are their slow and difficult growth, and the formation of minute transparent colonies with a relatively wide area of hemolysis. The original strains of Bliss and Long were obtained rarely from the throats of normal individuals and more often from patients with infections of the upper respiratory tract. In addition to the two Group F strains observed in the present series, others have been obtained from the tonsils of one patient and from the chest fluid of another patient in the Hospital of The Rockefeller Institute. The significance of these organisms in human infections is at present uncertain, al-though the two strains encountered in this study did not give rise to infection.

Group G.—This is another group not hitherto described. The immune sera used for its differentiation were made by the injection of rabbits with one of the strains studied here. The ten members of the group described in this communica-tion resemble the members of Group A very closely in their biochemical reactions, including the ability to digest human fibrin, a property which seven of the ten strains possessed. Two additional saprophytic strains from the vagina and six strains from normal human throats have also been encountered, as well as one strain from a case of otitis media in a dog and one from a fatal case of pneumonia in a monkey. Four strains from the throats of normal monkeys have also been found.

DISCUSSION

The researches described in this paper are, in effect, an attempt to differentiate those hemolytic streptococci which are likely to do harm during childbirth from those which either do not or cannot do so.

[6] It is improbable that the pseudohemolytic streptococci from the respiratory tract described by Cumming (19) are the same organisms. None of his strains fermented mannite, whereas all the Group D strains do so. After four sub-cultures none of the strains studied by Cumming showed hemolysis on rabbit blood agar plates although they were hemolytic on human blood agar. Further-more, his strains were indistinguishable, microscopically and colonially, from *S. pyogenes*; Group D strains can, with a little practice be readily distinguished.

The precipitin tests show that the vast majority of strains from definite infections of the uterus are members of Group A. Lancefield (7) has also described a group of 21 strains mainly from infections of the human respiratory tract which gave precipitates with Group A sera. Their biochemical reactions were similar to those of the majority of the infective uterine strains described in this paper; that is, both groups failed to hydrolyze sodium hippurate or to grow on 40 per cent bile agar, but attained a low pH in 1 per cent glucose broth, digested human fibrin, and fermented trehalose, lactose, and salicin and, very occasionally, sorbitol and mannite. Although it is possible that other groups may later be found implicated as producers of human disease, as suggested particularly by the recent work of Long, Bliss, and Walcott (22), it appears at present that Group A strains are the hemolytic streptococci most likely to cause serious human infection.

The vast majority of hemolytic streptococci from the birth canal which do not bring about active infections are not members of this group. Most of them fall either into Group B or D; the former being identical with certain strains causing bovine mastitis, and the latter resembling *S. faecalis* more than *S. pyogenes*. These two groups were differentiated by Hare and Colebrook (6) by means of biochemical tests, and this differentiation has been here confirmed serologically. The remaining non-infective strains fell into Group C, F, or G, or were unclassified. In their biochemical reactions they resemble Group A, and for this reason Hare and Colebrook were unable to differentiate them from the infective strains. But that they differ immunologically from Group A strains can hardly be doubted in view of the results of the present study. It would therefore appear that the differentiation, by a comparatively easy precipitin test, of hemolytic streptococci which are potentially infective from those which are harmless to man is entirely feasible.

It seems highly probable that the human nasopharynx is the main reservoir of Group A strains in nature. Because of this, and because of the great rarity of Group A hemolytic streptococci in the normal vagina, *ante partum*, there can be little doubt that the vast majority of puerperal hemolytic streptococcal infections are due to inoculation from some other source than the patient's genital tract and prob-

ably arise from the above mentioned reservoir in the patient or attendants. Thus, indirectly, this work confirms that of Smith (23) and of Paine (24), who showed that the organisms in the majority of hemolytic streptococcal infections of the uterus could be identified by agglutinin absorption as the same as those present in the nose or throat of one or other of the attendants at the time of delivery.

The correlation between puerperal infection and the presence of Group A hemolytic streptococci in the vagina, on the one hand, and the corresponding lack of serious infection though hemolytic streptococci of other serological groups are often present, is set forth in the following summary.

TABLE VI

Summary of Results with Reference to Group A Hemolytic Streptococci

Source of cultures	No. of cases with hemolytic strepto- cocci	No. of Group A strains isolated	Type of infection
Cases admitted to Isolation Block after delivery: 45	45	44*	All severe puerperal infec- tion
Women delivered in Hospital (cultures taken post partum): 837	85	1 0 1	1 fatal puerperal infection 18 minor infections 65 afebrile cases
Women delivered on District (cultures taken ante partum): 855	13	0	1 minor infection 12 afebrile cases

* A Group G strain, together with a *Staphylococcus aureus*, was isolated from the remaining case.

It is, therefore, evident that hemolytic streptococci may be harbored in the birth canal either before or after delivery, without causing disease, provided they belong to serological groups other than Group A. Group A hemolytic streptococci, on the contrary, are usually absent from the vagina ante partum or are exceedingly rare, as shown by the failure to find organisms of this group in cultures taken before delivery from the present series of patients. However, Group A hemolytic streptococci if present in the vagina post partum almost always give rise to serious puerperal infection. According to these results, therefore, Group A strains are probably the only hemolytic

streptococci capable of causing definite puerperal infection in the human species, and such infection almost invariably occurs if Group A hemolytic streptococci are present in the vagina.

CONCLUSION

1. The majority of strains of hemolytic streptococci from puerperal infections of the uterus were identified serologically as members of the Group A described by Lancefield.

2. The majority of strains isolated from the birth canal of women whose puerperium was afebrile were not members of Group A.

3. The existence of two new serological groups of hemolytic streptococci, Groups F and G, is described.

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