FURTHER STUDIES ON IV-VARIANTS OF SALMONELLA TYPHI-MURIUM (ÆRTRYCKE) WITH SPECIAL REFERENCE TO CULTURES FROM PIGEONS¹

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Landsteiner and Levine (1932) were the first workers to note that certain cultures of Salmonella typhi-murium were devoid of antigen V of the Kauffmann-White schema. They noted this deficiency in the Binns strain of Schütze (1920). Kauffmann (1934) examined 256 cultures of S. typhi-murium and found 16 of them lacking in antigen V. The cultures exhibiting this peculiar deficiency in their somatic antigens, and designated by Kauffmann as S. typhi-murium var. copenhagen, are commonly referred to as IV-variants. Their antigenic formula is IV XII: i : 1, 2, 3 instead of IV V XII : i : 1, 2, 3 which represents typical strains of S. typhi-murium. Zahn (1935) found one IV-variant in 74 cases of S. typhi-murium infection.

All the IV-variants described by the investigators cited above were isolated from human sources. Since it is well known that S. typhi-murium infection in man almost invariably has its origin in some animal reservoir of infection, it is important from an epidemiologic as well as an academic standpoint that the zoological distribution of the IV-variants be determined. Edwards (1935) reported that six cultures of S. typhi-murium isolated from pigeons belonged to the IV-variety. Hoffmann and Edwards (1937) reported the presence of IV-variants in an infection of rabbits. The rabbits were housed in hutches which

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adjoined pens containing pigeons infected with the same organism. Except for these observations, there are no reports of the occurrence of the organisms in the lower animals. The purpose of the present paper is to present further observations on the occurrence of IV-variants in animals.

MATERIAL AND METHODS

The material to be reported consists of 155 cultures of *S. typhi-murium* of animal origin. The sources of the strains are as follows: horses, 14; sheep, 2; guinea pigs, 14; rats, 3; mice, 6; turkeys, 40; chickens, 10; ducks, 17; canaries, 11; pigeons, 35; rabbits, 3. These cultures have all been subjected to antigenic analysis. Particular attention has been paid to the somatic antigens in order to determine the presence or absence of factor V in the somatic complex. The flocculating antigens were examined by the method used by Edwards (1936). The somatic antigens were examined by the method of Kauffmann (1934).

RESULTS

It may be said at the outset that no IV-variants were found among the cultures from horses, sheep, guinea pigs, rats, mice, turkeys, chickens, ducks or canaries. Their occurrence was limited to the strains from pigeons and rabbits. On the contrary the 38 cultures from pigeons and rabbits were all IV-variants, no strains having the typical *S. typhi-murium* somatic complex being found among these cultures. Thirty-three cultures from pigeons were derived from 12 different flocks located in Connecticut, New York, New Jersey, South Carolina, Kentucky, Ohio, Colorado and California. Two cultures were of German origin. These were received from Dr. W. Herrmann of Essen.

It has been found by Kauffmann (1934) and Edwards (1935) that cultures of *S. typhi-murium* which lack factor V are likely to possess biochemical characteristics that are not typical of *S. typhi-murium*. Among the 38 cultures studied here there were 9 which failed to ferment maltose. Sixteen strains gave negative Bitter tests. Eight failed to utilize ammonium salts when tested
by the method of Hohn and Herrmann (1936). Four of the cultures were anaerogenic. The fermentation of maltose has no epizootiological significance since maltose-positive and maltose-negative strains may be isolated from the same flock. Further, by cultivation in maltose broth and plating on Endo’s agar containing maltose, it is possible to obtain maltose-fermenting sub-strains from cultures which fail to ferment maltose. Red daughter colonies appear on the plates. These ferment maltose rapidly. It has been found, however, that all strains from the same flock give identical results in the Bitter test. Also the 4 anaerogenic cultures comprised all the strains from one flock.

DISCUSSION

The IV-variants of *S. typhi-murium* form a definite entity. While they may be looked upon as a loss variant of typical cultures of the species, they are quite constant in their antigenic composition. Kauffmann (1934) noted no variation in the IV-variants he examined. The writer has maintained some of these strains as stock cultures for more than 3 years. During this time no changes have been noted in the antigenic characters of the bacilli. Emanuel (1936) was not able to induce any change in the somatic antigens although he used procedures that have induced antigenic variation in other genera.

Since *S. typhi-murium* is so widely distributed throughout the various species of domestic animals, it is surprising to find the zoological distribution of the variants so restricted. In the present study the organisms were found only in pigeons and rabbits. The rabbit strains are those reported by Hoffmann and Edwards (1937). Inasmuch as the rabbits were housed adjacent to pigeons carrying bacilli of identical antigenic and biochemical characters, these strains undoubtedly should be considered as originally of pigeon origin. The presence of these strains in rabbits demonstrates that the variants may be transferred from pigeons to other animal species.

Hohn and Herrmann (1936) and Herrmann (1936) have classified the *Salmonella* group according to the ability of the bacilli to utilize ammonium salts as a sole source of nitrogen. They
have advanced the theory that those cultures which fail to utilize ammonia are much more restricted in their host relationships than strains which are able to grow in a medium containing ammonium salts as the sole source of nitrogen. Among the organisms which fail to utilize ammonia are placed such organisms as *Eberthella typhosa* and *Salmonella paratyphi A* which ordinarily parasitize only man. On the other extreme are placed such ubiquitous species as *Salmonella typhi-murium* and the Newport type which readily utilize ammonia. Herrmann (1936a) states that IV-variants of *S. typhi-murium* which he has isolated from pigeons in Germany fail to grow on the synthetic medium of Hohn and Herrmann. The writer has confirmed Herrmann's observation, using the two German cultures available. The cultures isolated in the United States vary in their ability to utilize ammonia. Of the 36 American strains studied, 28 grew readily on the medium of Hohn and Herrmann when either glucose or citrate was used as a source of carbon. The remaining 8 cultures failed to develop. The recent work of Kauffmann (1936) on single-colony isolations casts doubt on the value of ammonia utilization as a differential criterion.

It was found by Edwards (1936) that the cultures placed in the Binns type by White were antigenically identical with the pigeon cultures and with *S. typhi-murium* var. *copenhagen* of Kauffmann. Thus it is known that such variants are capable of producing disease in man. Likewise, it is well known that food poisoning may be caused by the consumption of pigeons or the eggs of pigeons infected with *S. typhi-murium*. The observations reported here demonstrate that the variants are enzootic in pigeons in the United States. The isolation of IV-variants from the human beings indicates that the infection was contracted either directly or indirectly from pigeons.

**CONCLUSIONS**

In a study of 155 cultures of *Salmonella typhi-murium* of animal origin it was found that the only IV-variants encountered were isolated from pigeons and from rabbits which had contact
with infected pigeons. Thirty-five cultures from pigeons all belonged to the IV-variety. This type is enzootic in pigeons in the United States. The occurrence of IV-variants of *Salmonella typhi-murium* in food poisoning indicates that the infection was contracted directly or indirectly from pigeons.

REFERENCES


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**ADDENDUM**

Since the above paper was prepared there has come to the attention of the writer a further paper by Hohn and Herrmann (Die Typen des Breslau bacteriums. Z. f. Hyg., 1937, 119, 369–382). In this paper the authors report the study of eleven strains of *S. typhi-murium* from pigeons. All these strains were IV variants. Three grew well in the medium of Hohn and Herrmann, while eight failed to grow. They conclude that failure
to utilize ammonium salts indicates that the organisms are of pigeon origin since they have not found this type in other animal species. They do not regard the lack of factor V as significant as they found one strain from a mouse and one strain from a calf which were also lacking in factor V. Nevertheless it seems significant that all of their pigeon strains were IV variants while only two strains from other species belonged to the variant type.