isolated from the stools of normal human carriers. All of the cultures fermented glucose, arabinose, xylose, rhamnose, trehalose, dulcitol, mannitol, and sorbitol with the production of acid and gas within 24 hours. Four cultures fermented cellobiose after 7 to 12 days incubation. One culture fermented inositol promptly; the other 4 cultures did not attack this substance. Lactose, sucrose, salicin and raffinose were not fermented by any of the cultures. Three strains utilized *d*-tartrate; 2 did not. All the cultures utilized *l*-tartrate, *i*-tartrate, mucate and citrate. H_2S was produced but indol was not formed nor was gelatin liquefied.

The cultures were agglutinated to titre by O serum derived from *S. newport* (VI, VIII) and removed all agglutinins from the serum. They were monophasic and their H antigens were related to those of *S. cerro* (XVIII:z_4, z_{23}...) of Hormaeche and Peluffo[4] and *S. duesseldorf* (VI, VIII:z_4, z_{24}...) of Hohn.[5] The relationships of the H antigens of the 3 types are set forth in Table I. From the results it is evident that the 3 types have distinct antigens. It is further evident that factors z_{23} (*S. cerro* serum absorbed by *S. duesseldorf*) and z_{24} (*S. duesseldorf* serum absorbed by *S. cerro*) of Kauffmann[6] must now be redefined, since *S. tallahassee* is agglutinated by both. It is also necessary to assign a symbol (z_{32}) to the specific fraction of *S. tallahassee*. Therefore, z_{23} must now be prepared by the absorption of *S. cerro* serum with *S. tallahassee*, z_{24} by the absorption of *S. duesseldorf* serum with *S. tallahassee* and z_{32} by the absorption of *S. tallahassee* serum with *S. cerro* and *S. duesseldorf*. The diagnostic formula of *S. tallahassee* is VI, VIII:z_4, z_{32}. . . .

Summary. Three new Salmonella types were described: *S. richmond* (VI, VII: y—1,2,3...), *S. daytona* (VI, VII:k—1,6...) and *S. tallahassee* (VI, VIII:z_4, z_{32}...). Attention was directed to the necessity of redefining factors z_{23} and z_{24} since *S. tallahassee* was agglutinated by both serums as they previously were prepared.

[4] Hormaeche, E., and Peluffo, C. A., *Arch. Urug. de Med., Cir. y Espec.*, 1941, **19**, 125.

[5] Hohn, J., *Z. f. Hyg.*, 1936, **117**, 722.

[6] Kauffmann, F., *Die Bakteriologie der Salmonellagruppe*, Copenhagen, 1941.

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Flagella and Flagellar Antigens in "Non-Motile" *Salmonella* Cultures.*

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During the 25 years that antigenic analysis has been applied to the classification of *Salmonella*, it gradually has become an accepted view that flocculating, heat-labile (H) antigens were associated with flagella and motility. Conversely, nonmotile microorganisms are generally considered to be devoid of flagella and H antigens. However, occasional references to the presence of H antigens in nonmotile strains can be found. Kauffmann[1] described a nonmotile culture of *S. aberdeen* which flocculated well in i serum and which was used to prepare the diagnostic i serum distributed by the International Salmonella Center. Such findings naturally raise a question as to whether H antigens are

* The work reported here was in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director. It was supported in part by a research grant from the U. S. Public Health Service.

[1] Kauffmann, F., *Acta Path. et Microbiol. Scand.*, 1939, **16**, 278.

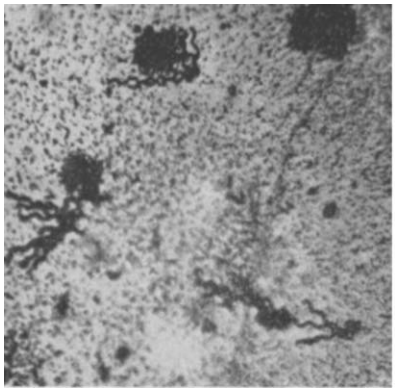

Fig. 1.
Flagella of *S. sandiego* strain. Van Ermengem's flagella stain. × 2000.

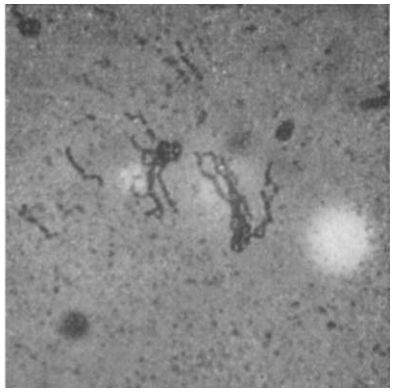

Fig. 2.
Flagella of III, XV:e,h strain. Van Ermengem's flagella stain. × 4000.

necessarily associated with flagella.

Several "nonmotile" *Salmonella* strains which flocculated with H serums were studied by the writers. One of these was a culture of *S. sandiego* received from Major R. H. Broh-Kahn. It was isolated and typed in the Central Diarrhea Control Laboratory of the Army Air Forces where it was recognized that although the culture possessed a normal complement of H antigens, it was nonmotile. Among nonmotile cultures typed in this laboratory, 3 had the formula VI, VIII:d and one was III, XV:e,h. The VI, VIII:d cultures were accidentally discarded but the *S. sandiego* and the III, XV:e,h strains were examined for flagella by the methods of Casares-Gil and of Van Ermengem. Cultures of actively motile bacteria, and of nonmotile bacteria which seemed to contain no H antigens were used for comparison. Large numbers of flagella were demonstrated in preparations from the motile cultures and from the nonmotile cultures which flocculated with H antigens. No flagella were observed in nonmotile cultures which failed to react with H serums. Photographs of flagella on the *S. sandiego* strain and the III, XV:e,h culture are illustrated respectively in Fig. 1 and 2. No differences were detected in the numbers or appearance of flagella present in the motile cultures and the flagellated nonmotile organisms.

The motility of the strains in question was judged by microscopic examination of broth cultures and by growth in stab cultures in the semisolid medium of Edwards and Bruner.[2] The VI, VIII:d type and the *S. sandiego* type exhibited no evidence of motility in serial transfers in semisolid medium over a period of several weeks. The III, XV:e,h bacteria began to migrate slightly from the site of inoculation after 3 serial transfers in semisolid medium, so that the growth had a rather beaded, fuzzy appearance. After 6 serial transfers it migrated slowly through the medium, producing an even turbidity. Behavior similar to that of the latter culture is not highly unusual in *Salmonella* cultures. However, such cultures usually do not flocculate with H serums until a fair degree of motility has been attained.

These observations constitute a confirmation of the results of Stuart and Wheeler[3] who, in their work on motility and swarming in paracolon cultures, noted flagella and H antigens on organisms which appeared nonmotile. They also noted that certain strains assumed motility after many months of growth in semisolid medium.

Two points are emphasized by the results presented here and by those of Stuart and Wheeler.[3] First, bacteria which appear nonmotile by accepted methods of examination may possess well-developed flagella and H antigens. This is added evidence of the

[2] Edwards, P. R., and Bruner, D. W., *Ky. Agric. Exp. Sta. Cir.* 54, 1942.

[3] Stuart, C. A., and Wheeler, K. M., 1946, personal communication.

flagellar nature of heat labile, flocculating antigens. As yet, no satisfactory explanation can be offered for the nonfunctional nature of the flagella in flagellated microorganisms which give no evidence of motility. Second, some degree of discretion should be exercised in characterizing a culture as being nonmotile. Unless a culture belongs to a well-known type which is known to lack motility, it should be examined carefully and be transferred serially in semisolid medium before it is concluded that motile forms cannot be obtained from it.

Summary. Two apparently nonmotile strains possessed well-developed flagella and flagellar antigens. One of the types (III, XV:e,h) eventually yielded motile elements after serial transfer in semisolid medium. The other *(S. sandiego)* gave no evidence of motility after similar treatment.

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Submerged Growth of Tubercle Bacilli from Pathologic Material in Dubos' Medium.

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The recent reports by Dubos[1] describing the remarkably rapid growth of Mycobacteria in a fluid medium containing purified phosphatides or certain synthetic nonionizing surface-active agents suggested the possibility that such media might be useful in the examination of pathologic material in the diagnostic laboratory.

During a period of 3 months 165 specimens were received for routine examination for tubercle bacilli by guinea pig inoculation. These specimens, untreated, were injected into guinea pigs according to the method in routine use. One hundred and forty-three of these specimens were examined by culture as well as guinea pig. For cultural examination, 5-20 ml volumes of the specimen were digested with an equal volume of 3% hydrochloric acid for periods varying from 15 minutes to 2 hours at room temperature, then neutralized to approximately pH 7.0 with 3% sodium hydroxide.[2] The digest was centrifuged and the sediment concentrated to 1/10th to 1/20th the original volume in sterile physiologic saline solution.

Several variations of basic media as described by Dubos[1] were used in this study (Table I). The various substances added to the basic media, together with the number of specimens examined in each are summarized in Table II. The specimens recorded as cultured in each basic medium were cultured in all the variations noted in Table II. Asolectin was first dispersed in a few ml of ether and added to the basic medium before autoclaving. Basic media were adjusted to pH 7.0-7.2 with normal sodium hydroxide and autoclaved at 15 lb. for 15 minutes. Glucose, albumin and Vegex were added to the autoclaved base in the form of sterile solutions. The media were dispensed in ordinary Pyrex test tubes 18-20 mm in diameter, stoppered with nonabsorbent cotton and sterilized in the autoclave. The various media were used in volumes ranging from 1 to 5 ml. Although growth can be obtained in as little as 1 ml of media, larger volumes (3-5 ml) were preferable since many cultures were kept in the incubator for 14-21 days, resulting in considerable reduction in volume by evaporation. Each lot of media was tested for growth-supporting capacity with stock strains of human (H37Va) and bovine tubercle bacilli and an acid-fast saprophyte *(M. phlei)* before

[1] Dubos, R. J., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 361.

[2] MacNabb, A. L., *Diagnostic Procedures and Reagents*, Am. Pub. Health Assn., 1941, Ed. 1, 281.