

Provisional *Shigella dysenteriae* 8 (*),

BY

EWING, W. H., VANDEPITTE, J., FAIN, A. and SCHOETTER, M.

(Received for publication : October 7, 1952.)

In this paper we wish to characterize and describe the relationships of an additional *Shigella* serotype and to propose its addition to subgroup A, the *Shigella dysenteriae* subgroup, as provisional *Shigella dysenteriae* 8.

At present, there are nine cultures of the new serotype 599-52, all of which were isolated in Belgian Congo (table 1). Eight of the cultures were isolated from febrile patients with dysentery whose stools were fluid and contained gross blood, mucous, and pus cells. One culture was isolated from a diarrheic stool of a patient with minor symptoms.

The biochemical reactions given by the nine cultures of serotype 599-52 were quite uniform. Acid, but not gas, was produced within 24 hours from glucose, maltose, arabinose, xylose, and trehalose by all cultures. Two cultures produced acid from rhamnose, one within 24 hours, the other after 5 days incubation. Seven cultures did not utilize rhamnose. Lactose, sucrose, mannitol, dulcitol, raffinose, sorbitol, adonitol, and salicin were not fermented. The cultures were nonmotile, did not grow on Simmons' citrate agar, hydrolyze urea, or produce acetylmethyl-carbinol. Nitrates were reduced to nitrites, the methyl red test was positive, and indol was formed.

The 0 antiserums used in this work were prepared by injection of broth cultures that were heated at 100 C for 2-1/2 hours, according to the procedures outlined by Kauffmann (1947, 1951). Suspensions used for absorption of agglutinin from 0 antiserums also were heated at 100 C for 1 hour as were the broth cultures

(*) From the Communicable Disease Center, Public Health Service, Federal Security Agency, Atlanta, Ga., the Laboratories of the Institute of Tropical Medicine, Leopoldville, Belgian Congo and the Laboratory of Astrida at Ruanda-Urundi.

used for 0 antigens in tube agglutination tests and thick suspensions for slide agglutination tests. Tube agglutination tests were incubated in a water bath at 50° C for 16 to 18 hours. None of the cultures used in the study contained alpha antigen (Stamp and Stone, 1944) nor was agglutinin for this antigen demonstrated in any antiserum employed in the work.

Cultures of serotype 599-52 were not related serologically to any of the known *Shigella* serotypes. Negative results were obtained when thick suspensions made with cultures of serotype 599-52 were tested in slide tests with antisera prepared with all members of subgroups A, B, C, and D of the genus *Shigella*, with provisional *Shigella boydii* 8, 9, 10, and 11 with four provisional shigellae (Ewing *et. al.*, J. Immunol., in press), and with serotype 2050-52, an undescribed bacterium isolated in Belgian Congo. However, the 0 antigens of serotype 599-52 cultures were found to be closely related to certain *Escherichia coli* 0 antigen groups, as is the case with many other *Shigella* serotypes. As shown in table 2, the 0 antigens of serotype 599-52 cultures were closely related to those of *E. coli* 0 groups 23 and 38 and, to a lesser extent, to those of *E. coli* 0 groupe 80. These 0 antigenic relationships are explainable by the use of the following arbitrary formulae (*).

Serotype 599-52	a, b, c
<i>E. coli</i> 038	a, b
<i>E. coli</i> 023	a, d
<i>E. coli</i> 080	(b), e

In the above formulae the symbol a was used to designate the antigenic factor that caused cross agglutination of cultures of serotype 599-52, *E. coli* 0 group 23, and *E. coli* 38 (table 2). In agglutinin absorption tests, all agglutinin was removed from *E. coli* 0 group 38 antiserum when it was absorbed with a suspension of serotype 599-52, while in the reciprocal absorption test a factor labeled c remained in serotype 599-52 0 antiserum. When 0 antisera for *E. coli* 0 groups 23 and 38 were absorbed reciprocally, the factor common to the two (a) was removed, but an agglutinative factor remained in each antiserum. The factor that remained in *E. coli* 0 group 23 antiserum was labeled d and the factor in *E. coli* 0 group 38 antiserum was labeled b. Thus, it was shown that the *E. coli* 0 group 38 culture had at least two 0 antigen fractions, a and b. That both of these factors were present in

(*) These arbitrary formulae are used merely as an aid to make antigenic relationships clearer and should not be interpreted as permanent designations for the antigens involved.

cultures of serotype 599-52 was demonstrated by the removal of all agglutinin from 0 group 38 0 antiserum when it was absorbed with a culture of serotype 599-52. That factor *d*, contained in *E. coli* 0 group 23, did not occur in serotype 599-52 cultures was shown by the fact that agglutinin for factor *d* remained in 0 antiserum for *E. coli* 0 group 23 following absorption with serotype 599-52. No 0 antigenic relationship was noted between cultures of *E. coli* 0 group 23 and 0 group 80. However, a relationship less extensive than those outlined above was noted among the 0 antigens of *E. coli* 0 group 38, *E. coli* 0 group 80, and serotype 599-52 cultures. The results of reciprocal absorption tests indicated that these relationships were caused by the presence of a part of factor *b* in the *E. coli* 0 group 80 culture (table 2). The letter *e* was employed in the arbitrary formula to indicate the major 0 antigen complex of *E. coli* 0 group 80.

The fact that the 0 antigens of serotype 599-52 cultures are related to those of *E. coli* 0 groups 23, 38, and 80 affords ancillary evidence that serotype 599-52 cultures constitute a new *Shigella* serotype, since other known *Shigella* serotypes are not related to these particular *Escherichia* 0 antigen groups (Ewing and Hucks, 1952).

Summary. — The biochemical reactions and serological relationships of nine cultures of serotype 599-52, a new *Shigella* serotype, are described. The nine cultures were recovered from nine cases of dysentery in Belgian Congo.

The 0 antigens of serotype 599-52 cultures are homogeneous and are unrelated to those of known shigellae, but are related to those of *Escherichia coli* 0 antigen groups 23, 38, and 80.

It is proposed that serotype 599-52 be added to subgroup A, the *Shigella dysenteriae* subgroup, as provisional *Shigella dysenteriae* 8.

Résumé. — Les auteurs décrivent les réactions biochimiques et les relations sérologiques de neuf cultures du sérotype 599-52, un nouveau sérotype de *Shigella*. Ces neuf cultures proviennent de neuf cas de dysenterie au Congo Belge.

Les antigènes 0 des cultures du sérotype 599-52 sont homogènes et non apparentés à ceux des *Shigella* connues. Ils sont toutefois apparentés aux antigènes 0 des groupes *Escherichia coli* 23, 38 et 80.

Les auteurs proposent d'ajouter le sérotype 599-52 au sous-groupe A, — le sous-groupe *Shigella dysenteriae*, — sous l'appellation provisoire de *Shigella dysenteriae* 8.

Samenvatting. — Beschrijving van de biochemische reacties en de serologische verwantschap van negen stammen van het serotype 599-52, een nieuw *Shigella-serotype*. Deze negen stammen werden afgezonderd in negen gevallen van dysenterie in Belgisch Congo.

De 0-antigenen van de stammen van serotype 599-52 zijn homogeen. Ze zijn niet verwant met de 0-antigenen van de gekende shigellae maar wel met deze van *Escherichia coli* groepen 23, 38 en 80.

Schrijvers stellen voor het serotype 599-52 toe te voegen aan de ondergroep A, — de *Shigella dysenteriae* ondergroep, — onder de voorlopige benaming van *Shigella dysenteriae* 8.

REFERENCES.

- Ewing, W. H., Hucks, M. C. Bact. Proc. Soc. Amer. Bact., 1952, 110.
Kauffmann, F. J. Immunol., 1947, 57, 71.
Kauffmann, F. Enterobacteriaceae, Ejnar Munksgaard, Copenhagen, 1951.
Stamp, L., and Stone, D. J. Hyg., 1944, 43, 266.

TABLE 1.
Sources of nine cultures of serotype 599-52.

Culture No.	Date Isolated	Place	Source
599-52	August 10, 1948	Leopoldville	Case with dysenteric stools. White female adult.
187-52	June 7, 1951	Logo (*)	Case with diarrhetic stools. Native male adult.
2116-52	December 17, 1951	Nioka (*)	Case with dysenteric stools. Native male adult.
2117-52	Februari 2, 1952	Nioka	Case with dysenteric stools. Native male adult.
2118-52	April 2, 1952	Nioka	Case with dysenteric stools. Native child of 1 year old.
3521-52	May 21, 1952	Nioka	Case with dysenteric stools. European adult.
4548-52	June 12, 1952	Nioka	Case with dysenteric stools. European adult.
4549-52	June 26, 1952	Niarembe (*)	Case with dysenteric stools. European child of 1 year old.
4550-52	July 7, 1952	Niarembe	Case with dysenteric stools. Native child of 3 years of age.

(*) These villages are located in the region of Ituri, in northeastern Belgian Congo.

TABLE 2.

The O antigenic relationships of serotype 599-52 and *E. coli* O antigenic groups.

O Antiserums	O Antigen Suspension (100 C., 1 hr.)			
	Serotype 599-52	<i>E. coli</i> 023	<i>E. coli</i> 038	<i>E. coli</i> 080
Serotype 599-52 :				
Unabsorbed... ..	20,480	5,120	10,240	1,280
Absorbed by :				
<i>E. coli</i> 023	10,240 (*)	0 (**)	2,560	1,280
<i>E. coli</i> 038	2,560	0	0	0
<i>E. coli</i> 080	10,240	5,120	5,120	0
<i>E. coli</i> 023 :				
Unabsorbed... ..	640	5,120	640	0
Absorbed by :				
Serotype 599-52	0	2,560	0	
<i>E. coli</i> 038	0	2,560	0	
<i>E. coli</i> 038 :				
Unabsorbed... ..	5,120	5,120	5,120	80
Absorbed by :				
Serotype 599-52	0	0	0	0
<i>E. coli</i> 023	2,560	0	2,560	80
<i>E. coli</i> 080 :				
Unabsorbed... ..	2,560	0	40	20,480
Absorbed by :				
Serotype 599-52	0	0	0	20,480

(*) Figures indicate highest dilution that gave strong agglutination.

(**) Zero indicates no reaction in lowest dilution tested (1:40).