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*Continuous (Enteric) fevers and meningitis due to infections with rarer strains of Salmonella Organisms

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Typhoid fever is to be accepted as being one of the oldest diseases. Hippocrates as well as several medieval authors have described a clinical picture closely related to the today's conceptions. It was, of course, not only typhoid, which was being described, but diseases appearing with a similar clinical picture may also have been included.

Since the development of bacteriology by Pasteur and Koch and the progress in protozoological work, the circle got narrower, as many diseases of a similar clinical appearance, e.g. relapsing fever, spotted fever (typhus) could be excluded by establishing their aetiology.

It was in the year 1880 that Eberth and Koch saw for the first time the germ causing typhoid fever in the spleen, mesenteric glands, liver, wall of bowel while performing post mortem examinations of typhoid corpses. But only in the year 1884 was Gaffky able to obtain the first pure culture, and later on to prove the existence of this bacillus in the sick bodies of patients suffering from typhoid.

About that time several other bacilli were isolated, causing typhoid-like diseases and were described as paratyphoid bacilli. But it took about twenty years before these bacilli could be strictly separated from the typhoid bacillus. First, of course, examination was done by cultural and biochemical methods only. Agglutination, absorption and cross agglutination to establish the antigenic structures have been introduced later. All these investigations lead to the diagnostic antigenic table, which is known today as the Kauffmann—White Schema.

International co-operation is essential in microbiological work to avoid errors, repetitions or double work. The International Association of Microbiologists dealing with this matter has, for common use of the same names, established a Committee of Nomenclature, which has established a particular sub-committee to deal with a group of typhoid-paratyphoid-enteric bacilli for which the name genus Salmonella was proposed for international use.

The above mentioned historical development can be followed by the definitions and recommendations of the Salmonella sub-committee of the last fifteen years.

'A large genus of serologically related, Gram negative and non-sporing bacilli; $0{\cdot}4\text{-}0{\cdot}6 \times 1\text{-}3\mu$ in usual dimensions, but occasionally short filaments; showing with certain exceptions a motile peritrichous phase in which they normally occur; in

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fact adhering to the pattern of B. typhosus in staining properties and morphology. Failing to ferment lactose and saccharose, to clot milk, to liquefy gelatin or to produce indole they regularly attack glucose with, but occasionally, without gas production. All the known species are pathogenic for man, animals or both '. (P. Bruce White, 1934, (1)).

An intensive investigation on Salmonella made it necessary in 1939 for the 3rd International Congress for Microbiology (New York) to propose some alterations.

' It is therefore proposed that the Bruce White definition be amended as follows :— Instead of Failing to ferment lactose and saccharose, to clot milk, to liquefy gelatin or to produce indole very regularly attack glucose, etc.—substitute—Failing to ferment sucrose and to clot milk and rarely fermenting lactose, liquefy gelatin or producing indole they regularly attack glucose, etc. This proposed modification of Bruce White definition stresses the fundamental importance of serological criteria in the classification of the Salmonella groups '.

This recommendation derived from Kauffmann's (2) proposal to base Salmonella diagnosis on serology only.

' Salmonella bacilli are Gram negative bacilli which due to their antigenic structure fit in the Kauffmann—White Schema '.

The latest investigations have lead F. Kauffmann (3) to classify the Enterobacteriaceae into four tribes : Salmonelleae, Eschericheae, Shigelleae, and Proteae. The tribe Salmonella is split into four groups as Salmonella, Arizona, Bethesda, Ballerup. The latter are assumed as being a linkage to the tribe Eschericheae.

The last issue of the Kauffmann—White Schema 1940 (4) enlists 142 recognised strains of the Salmonella group. But at the time there have been published several new strains of Salmonella, which are not yet officially enlisted, thus giving a steadily increasing number of Salmonella strains.

When looking at the Kauffmann—White Schema it is to be seen that we have to deal with three main types of antigens, i.e. the antigens of the bacterial body the somatic or ' O ' antigens, the ' Vi ' antigen which covers the bacterial body and suppresses ' O ' agglutinability, and the flagellar or ' H ' antigens. By the variety of these three antigens each species can be well defined.

The division into several groups is reached by the presence of common somatic antigens as for instance IV, V for group B or IX for group D, etc. Sub-division in each group is made by help of the flagellar antigens. An international Salmonella Centre at the State Serum Institute Copenhagen has been erected for the purpose of international co-operation in Salmonella research and establishing regional Salmonella Centres in important institutes of different countries. These regional Salmonella Centres are equipped with test strains to cover all Salmonella antigens and with the most important diagnostic sera. The Medical Research Institute, Colombo is acting as regional Salmonella Centre for Ceylon.

Now coming to the technical side, the most important materials to be checked for Salmonella are blood, faeces and urine. To ensure the highest possible rate of findings it is essential to have not only a direct plating but to inoculate media such as Kauffmann's combined enrichment medium (5), which enhance the growth of Salmonella organisms but suppress the growth of other mostly not pathogenic

bacteria, e.g. E, coli, Proteus, Ps. pyocyanea, etc. The essential ingredients of such media are bile, or bile salts. Tubes inoculated with blood are to incubated for 4-5 days with daily sub-cultures on ss-agar. For enrichment of faeces and urine it is sufficient to incubate 48 hours with daily sub-cultures on suitable media.

It must be taken as a basic mistake to omit the inoculation of enrichment media, as by this method an increase of positive findings up to over 500% is found compared with primary plating only.

Suspicious colonies are isolated and checked for fermentation, gas and H_2S formation in Kligler's double sugar-iron agar and for decomposition of urea. By this way organisms such as E, coli, Aerogenes faec., Ps. pyocyan. Proteus are ruled out quickly.

The remaining strains are checked serologically first with a polyvalent serum including the antigens representative for the group A to E. The reason for this procedure is based on Edwards and Bruner (6), Seligmann, Saphra and Wassermann (7), Bruner and Joyce (8) findings. They found these groups being representative of 98·3% to 99·5% of all Salmonella cultures isolated. Therefore this method was adopted for Salmonella Centres (9).

Cultures giving a positive agglutination are then checked with the particular group sera. Cultures negative in agglutination are checked for the presence of Vi-antigen, as this antigen suppresses ' O ' agglutinability.

It is quite easy to type S. paratyphi A and S. typhi, as the former is the only strain containing the somatic antigen II and the flagellar antigen a, the latter being the only strain of group D containing the antigens IX, Vi, and d. For all other strains it is essential to establish the antigenic formula by agglutination with single factors and proof of identity by cross absorption with the type strain of the same formula, e.g. S. virchow VI, VII r 1, 2.

The still remaining negative cultures are checked biochemically on a full set of carbohydrates and organic acids for typical behaviour of Salmonella strains. So far we have not found such a strain of Salmonella belonging to other groups, but we are investigating a few strains, which split late and irregularly lactose, saccharose or both and may belong to one of the above mentioned groups of the tribe Salmonella. These investigations are not completed as yet.

The sources of the specimens checked since June 1950 are listed in Table 1.

TABLE 1

Bloods	*Faeces*	*Rectal swabs*	*Urines*	*Cerebrospinal fluids*	*Other Specimens*	*Total*
4777	209	140	15	4	12	5157

The number of blood samples is roughly 13 times higher than all other specimens checked. About 33% of these blood samples had a positive Gruber Widal reaction and about 17% a positive blood culture. But nearly all faeces from these patients are missing ; that means these patients were discharged without cultural examination and many of them may be carriers. This shows clearly that the importance of cultural work on faeces, etc. has not yet been appreciated by all clinicians.

In this connection it must be stated that the distribution of Salmonella strains isolated from blood cultures is more representative than results obtained from faeces, the latter being results of a selected and comparatively small material.

The checking of specimens other than blood gave the following results :—

TABLE 2

Faeces	*Rectal Swabs*	*Urines*	*Cerebro-spinal fluids*	*Other Specimens*	*Total Positives*	*Total*
209	140	15	4	12	—	380
23 pos.	1 pos.	2 pos.	2 pos.	—	28	—
11%	0·07%	—	—	—	7·4%	—

These results, even obtained from a comparatively small number of specimens, confirm that faeces only are suitable for a successful checking, as the distribution of germs in the intestines is not uniform and rectal swabs, mostly taken from the very end of the intestines, do not contain other bacteria than the most common inhabitants as E. coli, Proteus, etc. or even are sterile.

The different types of the organisms isolated and their sources are classified in Table 3.

TABLE 3

Strain	*Blood*	*Faeces*	*Rectal Swabs*	*Urine*	*Cerebrospinal fluid*	*Total*
Group A 18·7%						
S. paratyphi A	151	1	—	—	—	152
Group B 0·4%						
S. paratyphi B	1	1	—	—	—	2
S. san diego	—	1	—	—	—	1
Group C 2·5%						
S. virchow	2	15	—	2	—	19
S. tennessee	1	—	—	—	—	1
S. kentucky	1	—	—	—	—	1
Group D 78·3%						
S. typhi	629	3	1	—	—	633
S. enteridis	1	2	—	—	2	5
Group E 0·1%						
S. muenster	—	1	—	—	—	1
Total	786	24	1	2	2	815

In discussion of Table 3, there is nothing to be mentioned concerning S. paratyphi A and S typhi. These strains are isolated the world over.

Infections with Salmonella Group B strains are relatively rare in East Asiatic countries (5) and have been isolated by us three times (0·4%) only. Two of these strains were S. paratyphi B, a well known representative of this group, whereas the third strain, S. san diego, has been isolated from an outbreak of food poisoning in 1938 and since this time it was found several times in man and pigs, which may be the animal reservoir.

Infections with Salmonella Group C strains have been found rather more frequently. It is to be mentioned that three strains of them have been isolated from two healthy carriers (S. virchow).

Most of these strains are S. virchow. It was isolated first in 1927. Since this time S. virchow has been found to be responsible for several cases of gastroentritis.

S. tennesse was isolated first in 1942 from an outbreak of food poisoning and later found in man, fowls and dried egg powder.

S. kentucky was isolated in 1937 from chicken. This strain was later found to be responsible for gastroenteritis in man, and was isolated from camels too. This is the second time it has been isolated from blood (10, 11).

S. enteritidis was the only type isolated other than S. typhi belonging to Group D. It was isolated twice from Cerebrospinal fluids from children suffering from meningitis. One patient, a fatal case, excreted the identical strain in faeces too, the other child which recovered was positive in Cerebrospinal fluid only.

Children are very prone to infections with Salmonella strains, especially in the developing septicaemia enteritidis strains. This higher susceptibility of children may also explain the higher incidence of group C infections, as nearly all the group C strains have been isolated from children's faeces.

The 4th and 5th strains of S. enteritidis have been isolated from faeces as mixed infection with S. paratyphi A and from the blood respectively. The specimen of faeces was sent for culture for M. tuberculosis, thus the patient being a carrier of two Salmonella strains.

Only one strain belonging to Group E, S. muenster, has been isolated. S. muenster was isolated in 1934 from an outbreak of food poisoning, and later it was found to be responsible for gastroenteritis and infantile diarrhoea.

As all these strains of Salmonella belong to the food poisoning group, care should be taken to eliminate carriers amongst cooks and food handlers.

In addition to these findings of Salmonellae, 3 strains of Shigellae belonging to Sh. flexneri groups III, IV, VI (nomenclature according to Boyd (12)) have been isolated.

To lower the rate of Salmonella cases, care should be taken not to discharge patients without cultural examination of faeces and urine. We are fully aware that Hospitals are over crowded and checking three times with one week's interval is not possible at present. But it would be a big advantage to have at least one checking of faeces and urine during the time the patients stay in hospitals after defervescence, that means on the 5th day after defervescence. Then the report reaches the hospital not later, than the day of discharge and in case of positive findings the particular patient should not be discharged until negative results are obtained.

Summary

By using improved methods we have isolated, in addition to S. paratyphi A and S. typhi, organisms such as S. paratyphi B, S. san diego, S. virchow, S. tennesse, S. kentucky, S. enteritidis and S. muenster.

References

1. WHITE BRUCE, P., *J. Hyg.* 34, 344, 1934.
2. KAUFFMANN, F. Z., *Hyg.* 119, 352, 1937.
3. KAUFFMANN, F. Z., *Act. Path. Scand.* XXVI, 6, 1949.
4. KAUFFMANN, F. Z., *Act. Path. Scand.* XXIV, 3-4, 1947.
5. KAUFFMANN, F. Z., *Die. Bakteriologie der Salmonell groupe, Einar Munksgaard, Kopenhagen,* 1941.
6. EDWARDS, P. R and BRUNER, D. W., *Serological Identification of Salmonella Cultures Ky. Ager. Exp. Sta.* Circular 54, 1942.
7. SELIGMANN, E., SAPHRA, J. and WASSERMANN, M., *Salmonella injections in the U.S.A., J. Immunol* 54, 69, 1946.
8. BRUNER, D. W. and JOYCE, B. J., Salmonella Types encountered by the 15th Medical General Medical Laboratory., *A. M. J. Hyg.* 45, 19, 1947.
9. KAUFFMANN, F. and EDWARDS, P. R., *J. Lab. and Clin. Med.* 32, 5, 548, 1947.
10. EDWARDS, P. R., BRUNER, D. W. and MORAN, A. B., *J. Inf. Dis.* 83, 220, 1948.
11. KAUFFMANN, F., Personal Communication, 1950.
12. BOYD, J. S. K., *J. Path and Bacteriology,* 58, 237, 1948.