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Experimental Type Transformation of *Shigella paradysenteriae* (Flexner).

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Griffith and Dawson observed type transformation of pneumococci *in vivo* and *in vitro* induced by dead pneumococci or extracts of the transforming type. The transforming principle was shown by Avery and his collaborators to be a desoxyribonucleic acid (for references see[1]). Recently, Boivin[2,3,4] reported transformation of an *E. coli* strain induced by a filtrate strain of a different type. Again the inducing agent was identified as a desoxyribonucleic acid. The theoretical aspects of these observations have been discussed.[5]

Considerable information obtained in recent years on the antigenic properties of Shigellae, particularly *Shigella paradysenteriae* (Flexner)[6,7] provide a basis for an investigation of type transformation of *Shigellae*. During the last 2 years several attempts were made to obtain evidence of this kind. The first results were disappointing, but information worthwhile mentioning here was obtained: we found it difficult to produce true R variants of Flexner bacilli on a number of fluid and solid media, including synthetic ones[8] providing only minimal requirements for growth. Cultivation in homologous immune serum in concentrations up to 10% was also not conducive to the appearance of R variants. Bacteria grown in broth containing up to 10% of homologous or heterologous immune serum or in normal serum gave no indications of changes in their antigenic behavior. Also addition of formol-killed bacteria to nutrient broth of a heterologous type did not influence the cultural and serological properties of *Shigellae*. However, in the light of experiences reported below it cannot be excluded that the number of experiments with this technique was insufficient to disprove the possibility of obtaining type transformation with killed organisms as inducing agent.

However, we have been able to secure evidence of type transformation in Flexner bacilli with extracts similar to those employed by Boivin.

Technique. Cultures of *Sh. paradysenteriae* were grown in beef heart broth (without dextrose, pH 7.4) at 37°C for approximately 18 hours. Ten drops of toluene were added to each 40 ml of culture, which was kept at room temperature for 4 hours under repeated shaking. The bacteria were separated by centrifugation in an angle centrifuge placed in a chillroom (±4°C). The supernatant fluids of two 40 ml tubes were pooled and sent through a filter candle of medium porosity. The filtrates were stored in the chillroom and distributed in 3 ml volumes in small test tubes.

The filtrates were inoculated from cultures grown 18 hours at 37°C on beef heart agar slants. After incubation at 37°C overnight platings on beef heart agar were made. They were repeated one or several days later as desired. Sterility tests accompanied each step of the procedure, and an uninoculated tube was incubated and plated out together with the inoculated filtrates.

Strains from our collection whose stability in serological and cultural properties was known for at least one year were employed. They were examined anew before entering the experiments.

1 McCarty, M., *Bact. Rev.*, 1946, **10**, 63.

2 Boivin, A., Delaunay, A., Vendrely, R., and Lehoult, Y., *Experientia*, 1945, **1**, 334.

3 Boivin, A., Delaunay, A., Vendrely, R., and Lehoult, Y., *Experientia*, 1946, **2**, 139.

4 Boivin, A., and Vendrely, R., *Helvet. chim. acta*, 1946, **5**, 1338.

5 Luria, S. E., *Bact. Rev.*, 1947, **11**, 1.

6 Weil, A. J., Black, J., and Farsetta, K., *J. Immunol.*, 1944, **49**, 321 and 341.

7 Weil, A. J., *J. Immunol.*, 1947, **55**, 363.

8 Weil, A. J., and Black, J., Proc. Soc. Exp. Biol. and Med., 1944, **55**, 24.

Colonies isolated from platings were examined by the slide agglutination technique with absorbed sera.[6,7] If mutants were obtained, the colonies and, at the same time, non-mutated colonies were secured on agar slants and compared in test tube agglutinations and in cultural tests with the behavior of the parent strain and that of the strain from which the filtrate was derived ("inducing strain"). The following cultural methods were used in these comparative tests: beef heart agar for the observation of growth, colony form and microscopy, beef heart broth incubated both at room temperature and at 37°C, from which hanging drops were examined, appropriate media for tests of indol formation and of reduction of trimethylamine oxide, the decomposition of urea, the liquefaction of gelatin, and formation of acid and gas from dextrose, lactose, mannitol, maltose, dulcitol, rhamnose, arabinose, and salicin.

Filtrates were usually inoculated in groups of 6 with the corresponding strains, thus adding an additional safeguard by cross check. Altogether 38 filtrates involving Types I, II, II.VII, III, V, VI, VII, VIII, IX, and XII of the Flexner bacillus* were inoculated with at least 6 strains of different types. In 225 tests unequivocal evidence of type transformation was obtained in 3 cases, namely a filtrate of Type I inoculated with a strain of Type II.VII, a filtrate from a strain Type II inoculated with a strain of Type V, and a filtrate of a different strain Type II inoculated with a strain Type XII. In each case platings showed colonies both of the parent type and of the type of the inducing filtrate. In the first case, of 16 colonies examined, 10 were of Type II.VII and 6 of Type I; in the second case, of 40 colonies, 12 were of Type V and 28 were of Type II; and in the third case, of 95 colonies 49 were of Type XII and 46 of Type II. These figures were obtained from a single plating in the first case, and from 3 platings made on different days in the second and third case.

The Type V strain of the second case of transformation differed from the inducing strain of Type II, in that the Type V strain was indol positive whereas the Type II strain did not form indol. All colonies from the platings reacting serologically like the parent strain gave a positive indol reaction, whereas the mutated colonies had lost the ability to form indol. As it happened, the parent and the inducing strain differed in a second property namely, the Type V strain does not form acid from maltose, whereas the inducing Type II strain acidifies this carbohydrate within 24 hours. Both non-mutated and mutated colonies from the platings did not acidify maltose. We then have here a mutation of one biochemical property coupled with the transformation of the somatic antigen, whereas a second property remains unchanged, thus tagging the mutants according to their parentage.

In the 2 other cases of transformation, the parent and the inducing strain were indistinguishable in their cultural properties and therefore did not offer a chance to obtain additional data on the association of mutation of biological and serological properties.

The parent Type II.VII strain of the first mentioned case of transformation grows on agar in slightly more opaque colonies than those of the inducing Type I strain. In the cultures obtained from the filtrates, those of serological Type II.VII were more opaque than the mutant colonies of Type I. This is illustrated in Fig. 1 where the parent and the inducing strain and one non-mutated and one mutated culture were plated out on separate quadrants of an agar plate. The difference in opacity may conceivably be merely the visible expression of the change in antigens. But it is also possible that opacity depends on some physical property of the bacteria independent from the somatic antigen. There is at present no way to decide whether or not the differences in opacity indicate another associated mutation.

The mutant strains have now been under observations for 10, 8, and 2 months respectively. During this period they were repeatedly plated out and numerous single colonies were tested for evidence of reversion to the original type. No such evidence was obtained.

Hitherto we have been unable to reproduce

* For type designation see 6.

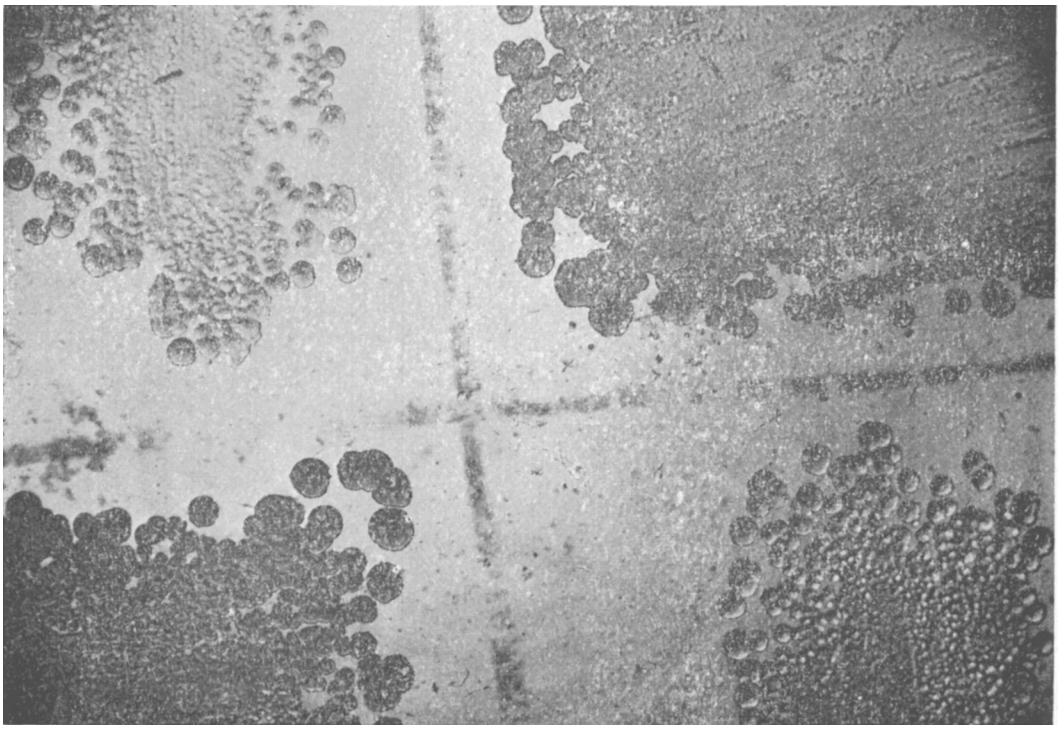

FIG. 1.
Upper right: parent strain (type II.VII).
Upper left: inducing strain (type I).
Lower left: subculture from non-mutated colony (type II.VII).
Lower right: subculture from mutated colony (type I).
Note: The apparent paleness of some of the upper left hand section is due to the circumstance that this is the point of maximal illumination.

at will the phenomenon described. That is, new filtrates made from the inducing strains did not evoke mutations from the strains with which we were successful the first time.

Discussion. In 3 out of 225 attempts evidence of induced type transformation of Flexner bacilli by culture filtrates was obtained. In the single case where the parent and the inducing strain were distinguishable in their cultural behavior, one such distinguishing property, namely that of indol formation, was coupled to the change in somatic antigen, whereas a second one, namely acid formation from maltose, was not correlated with the serological transformation. Our experience resembles that of Boivin[2,3,4] in two important points. Induced transformation could be produced in relatively rare instances —a single one in Boivin's case and 3 in ours. Moreover, Boivin found a mutation in a biochemical capacity (sucrose fermentation) related with the change in antigen just as we found the serological mutation associated with the property of forming indol.

In our experience, the type transformation was not visibly mediated by a transition into the R phase. The lack of visible evidence of the R phase preceding transformation may, but does not necessarily, indicate a difference in the mechanism of transformation of *Sh. paradysenteriae* and that of *E. coli* and pneumococcus. Further study of this point is clearly indicated.

We were less fortunate than Boivin in that we have not been able to reproduce transformation at will with a given filtrate and a given strain, while Boivin evidently could, as indicated by the fact that he could collect amounts of filtrates large enough to proceed with an isolation of the desoxyribonucleic

acid. Thus, the factors involved in the induction of type transformation of Flexner bacilli require further study both concerning the parent organisms and the properties of filtrates.

The demonstration of induced type transformation in *Shigellae* is not only of theoretical interest,[5] but it also cannot fail to influence our viewpoints on problems of the biology of the enteric flora. These have just been discussed at some length,[7] and it may suffice here to recall this aspect of induced type transformation.

Summary. Induced type transformation of *Sh. paradysenteriae* (Flexner) has been obtained in 3 out of 225 experiments. The inducing principle is contained in filtrates of broth cultures.

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Ovarian Hyperemia in the Immature Rat as a Pregnancy Test in Equids.

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Early diagnosis of pregnancy in equids by means of a biological reaction was studied by Cole and Erway.[1] A new method has been introduced by Zondek and Sulman[2] based on the ovarian hyperemia in immature rats injected with serum gonadotrophin. The same reaction proposed by Salmon *et al.*,[3] as a 6-hour test for human pregnancy, was challenged by Farris[4] and accepted with restrictions by Bunde.[5] Recently Zondek and Sulman[6] have recommended it again as a 24-hr test for the diagnosis of equine pregnancy. This note deals with the evaluation of the new test which was employed as a preliminary step for the preparation of mare gonadotrophin.

Experimental. Blood samples from 38 mares and 4 female donkeys obtained between 42 and 138 days after natural insemination were tested. Two infantile female rats weighing 40-55 g were injected subcutaneously with 1 and 2 cc respectively of each serum and killed 24 hours later. The ovaries were inspected in daylight and the response considered positive when they were enlarged and their color matched that of the kidney or the spleen. Pale yellow and small sized ovaries were indications of a negative response. Occurrence of pregnancy was assumed when one or both of the injected rats showed a positive response. With this technique and using rats from the same colony, positive reaction was always obtained 24 hours after the subcutaneous injection of 10 I.U. of a standard preparation of equine gonadotrophin. Therefore, when a positive response with 2 cc of serum was obtained, a blood concentration of at least 2,500 I.U. of gonadotrophin per liter was assumed to be present. Of course this is not an exact quantitative measurement but only a routine approximation.

A careful record was kept of each animal until evidence of pregnancy was established either by obvious abdominal enlargement or by subsequent parturition.

Results. Table I gives the results of the test with the serum of 38 mares and 4 female donkeys and the number of days between the natural insemination and the taking of

* Fellow of the J. S. Guggenheim Memorial Foundation.

1 Cole, H. H., and Erway, J., *Endocrinology*, 1941, **29**, 514.

2 Zondek, B., and Sulman, F., *Nature*, 1945, **3932**, 302.

3 Salmon, U. J., *et al.*, *J. Clin. Endocrinology*, 1942, **2**, 167.

4 Farris, E. J., *Am. J. Obst. and Gyn.*, 1944, **48**, 200.

5 Bunde, C. A., *Am. J. Obst. and Gyn.*, 1947, **53**, 317.

6 Zondek, B., and Sulman, F., *Endocrinology*, 1947, **40**, 322.