and the low requirement of the rat for this vitamin. It also appears plausible from analogous studies on vitamin K deficiency[9-12] that intestinal synthesis of the *L. casei* factor, known to occur,[13] may be a significant factor.

It should be pointed out that the number of cases of granulocytopenia noted in this series of rats does not necessarily represent the maximal incidence. Observations were not made over a long period of time in most rats, and the purified diets used are not considered to be completely free from "folic acid."

The absence of severe anemias in this series of rats does not prove that the erythropoietic system was unaffected. It has been shown that when sulfasuxidine-fed rats with normal hematocrit values are subjected to repeated bleedings, red blood cell regeneration may be very inadequate.[7] Similar studies have not been carried out as yet in a large series of rats fed purified diets without sulfonamides.

Summary. 1. Granulocytopenia was observed in a small percentage of rats fed purified diets without sulfonamides. 2 Crystalline *L. casei* factor ("folic acid") administered to 5 rats with granulocytopenia corrected the blood dyscrasia in every case. One or more relapses occurred in 3 of the rats. Crystalline *L. casei* factor ("folic acid") corrected the granulocytopenia in relapse.

[9] Dam, H., and Glavind, J., *Z. f. Vitaminforschung*, 1939, **9**, 71.

[10] Greaves, J. D., *Am. J. Phys.*, 1939, **125**, 429.

[11] Kornberg, A., Daft, F. S., and Sebrell, W. H., *J. Biol. Chem.*, 1944, **155**, 193.

[12] Kornberg, A., Daft, F. S., and Sebrell, W. H., *Pub. Health Rep.*, 1944, **59**, 832.

[13] Mitchell, H. K., and Isbell, E. R., *The University of Texas Publication*, No. 4237, 1942, 125.

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A Heretofore Undescribed Phase-Variation in Salmonella.

ERICH SELIGMANN, IVAN SAPHRA, AND MICHAEL WASSERMANN. (Introduced by Ella H. Fishberg.)

From the Department of Bacteriology and Serology, Beth Israel Hospital, New York City.

Andrews[1] discovered the phenomenon of *phase-variation* in bi-phasic Salmonella types. If a motile strain f.i. *S. typhi murium* (IV.V;i-1,2,3 . . .) is plated out on a moist agar plate, single colonies after 24 hours present either the i or the 1,2,3 phase. On further subculture either phase splits off the other phase in a certain percentage, generally not exceeding 5%. The graphic expression of this phase-variation reads: IV.V; i $\longleftrightarrow$ 1,2,3.

The two phases present in the two kinds of colonies are easily identified by known monophasic antisera or by antibody production. If a hitherto unknown phase appears and is not agglutinated by known antisera, it can be classified by immunization of rabbits with single colonies of this phase.

The unusual phase-variation which we are reporting differs from this pattern. A culture isolated from the blood stream of a severely sick elderly woman* showed culturally and serologically almost all the characteristics of *S. typhi murium*. The somatic antigen was IV.V, the flagellar antigen bi-phasic, one phase representing the 1,2,3 antigen. The other phase, however, was not the presumed i nor was it agglutinable by any one of our complete set of H-antisera. On occasion a trace of a 1,2,3 reaction was seen, suggesting that this phase, for simplification called X phase, may split off the 1,2,3 phase. Plated out from single colonies the X phase split off 1,2,3. The original 1,2,3 phase on the other hand split off

[1] Andrews, F. W., *J. Path. and Bact.*, 1922, **25**, 505.

* The patient died. Culture and clinical data were kindly submitted by Dr. Kantrowicz, Beth Moses Hospital, New York.

TABLE I.
Effect of Gard's Technic on the H-Antigens.

Phase	Serum applied	Direct result	Consecutive phase variation
1,2,3	1,2,3-antiserum	X phase	X ⟷ 1,2,3
1,2,3	i- ''	1,2,3 ''	1,2,3 ⟷ X
X	1,2,3- ''	i ''	1,2,3 ⟷ X (i)
X	i- ''	1,2,3 ''	1,2,3 ⟷ X
X	i-antiserum + 1,2,3-antiserum	immobilized X phase	X ⟷ 1,2,3

the X phase, both in a ratio of 25%. Thus, a phase-variation was observed, the X phase, however, remained inagglutinable also when derived from the 1,2,3. Both phases were motile, the X phase to a lesser degree. It was, however, not difficult to obtain well motile inagglutinable X phases in broth culture. Six-hour growth in broth, formolized, served as antigen for the immunization of rabbits. The resulting antiserum contained O-antibodies against IV.V and H-antigens. The latter were not of a new type; instead they represented a mixture of antibodies against the 1,2,3 and the heretofore missing i phase in almost equal amounts. Titers were: for O 1:1280, for i 1:6400; for 1,2,3 1:6400. The antigen of the X phase was agglutinated by this serum in granular form up to the serum's O titer 1:1280; no floccular (H) agglutination was observed. Absorption of the serum by normal *S. typhi murium* culture removed all antibodies and did not leave behind any substances reacting with the X phase. This phase, therefore, must contain the 2 typical H-antigens in almost equal amounts. That in itself is a rare incident, having been only once previously described in a culture of *S. paratyphi B* by Kauffmann.[2] But under the observed conditions our X phase cannot be agglutinated although it readily produces antibodies. In order to ascertain whether inhibiting substances were covering the H-antigens similar to the Vi in somatic antigens, recourse was had to sonic vibration.† No liberation of H-phases could be detected. Electron microphotography performed through the courtesy of Dr. G. Shwartzman of Mt. Sinai Hospital, N.Y., did not reveal any morphological differences between the X phase and the normal *typhi murium* culture.

As mentioned before the X phase splits off the 1,2,3 phase readily; spontaneous development of an i phase could not be obtained under the usual or changed growth conditions. Neither growing at different temperatures nor change of media—glucose broth, brain-heart infusion broth, ascitic broth, etc.—nor the shift of pH to the acid or alkaline side had any effect. Daily transplants were performed for 2 months and prolonged cultivation in the same broth was used without success. Ultraviolet radiation and repeated freezing and thawing were as unsuccessful as enteral or parenteral passage through mice. Successive transfers from mouse peritoneum to mouse peritoneum in 20 generations did not affect the X phase, not even a 1,2,3 phase developed in this prolonged experiment.

To summarize: Both the original phases (X and 1,2,3) spontaneously split into 1,2,3 and X individuals. In no instance, under greatly varied conditions, did an i phase develop.

Gard's technic of evoking the appearance of suppressed phases by addition of antiserum of the other phase was then applied to the two phases. The result of these experiments may be seen in Table I.

The effect of the Gard treatment on the X phase is twofold. Under the application of an anti-i serum a 1,2,3 phase is liberated, confirming the actual presence of this phase

[2] Kauffmann, F., *Zbl. Bakt.*, 1934, **132**, 20.

† The authors are indebted to Dr. David Green and Mr. P. K. Stumpf of Columbia University, College of Physicians and Surgeons, Department of Medicine, who kindly performed the sonic vibration.

previously found in spontaneous phase variation. Addition of an anti-1,2,3 serum, however, brought out the hitherto missed i phase. Combined treatment with i and 1,2,3 antiserum immobilizes the culture without appearance of any other phase. On subculture the "induced" i phase reproduces 3 phases, the i, X, and 1,2,3, the latter 2 forming about 5% of the colonies. The action of 1,2,3 serum on the original 1,2,3 phase sets the X phase free, the i antiserum has no influence.

Altogether, 3 well defined phases were recovered by this method: X, i, and 1,2,3. Later in animal experiments a fourth variant was found: a well agglutinable i + 1,2,3 phase in single colonies, comparable to that found by Kauffmann in *S. paratyphi B* (b + 1.2).

Living cultures of the original strain and of the various phases were unusually virulent for animals. Of 5 rabbits, one guinea pig, and more than 100 mice which were fed or injected, only one mouse survived. Among the fatalities was one rabbit which had been immunized previously with a formolized culture. The minimal fatal doses used consisted of 10^{-8} dilutions of broth, representing about 50 organisms. Computed on the average time of survival, the X phase exerted the highest pathogenicity in mice, the 1,2,3 phase the lowest. The number of animals involved in the study of each phase was not large enough to allow statistical evaluation of results. When mice were fed only the X phase, both X and 1,2,3 colonies were recovered from blood and stool. When the 1,2,3 phase was fed, again both X and 1,2,3 colonies were isolated from blood and stool. Of the six mice fed the "induced" i phase, five died, one survived, and the cultures yielded i, 1,2,3, and X colonies from the blood and feces. From the one surviving mouse pellets of feces were taken after 19, 41, 70, and 103 days. The first specimen showed only i colonies, the specimen secured on the 41st day yielded i colonies and colonies which surprisingly turned out to be bi-phasic, i + 1,2,3. The specimen collected on the 70th day displayed i, 1,2,3, and i + 1,2,3 colonies, and the one collected on the 103d day yielded i and i + 1,2,3 colonies. In animals and on subculture the i + 1,2,3 phase yielded the i, 1,2,3, and i + 1,2,3 phases but never the X phase. This i + 1,2,3 phase seen in about 10% of the single colonies, might actually be identical with the X phase which now became agglutinable. A formolized 6-hour broth culture of this variant was used for rabbit immunization. The resulting antiserum was active against the O (IV.V), i and 1,2,3 antigens. The fact that after removal of the O-antibody by specific absorption the remaining H-antibodies did not agglutinate the X phase indicates there is no difference between this serum and that produced against the X phase—the X phase itself has retained its inagglutinability now for 11 months.

Summary and Conclusions. The natural appearance of an inagglutinable but antigen-producing "mixed" phase of a *S. typhi murium* is described. The phase i, the presence of which could not be elicited in the original cultures, was brought out by the use of Gard technic. An agglutinable "mixed" phase was produced *in vivo*. Data on the different phase variations of this Salmonella variant are given.

The described observations raise a number of theoretical questions unanswerable for the time being. This paper, therefore, limits itself to a statement of the facts.

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A New Salmonella Type: *S. Virginia.*

IVAN SAPHRA AND ERICH SELIGMANN. (Introduced by Ella H. Fishberg.)

From the Department of Bacteriology and Serology, Beth Israel Hospital, New York City.

Culture 2255 was received on October 10, 1944, from A. Corpening, Director of Laboratories, Department of Health, Richmond, Va., where it had been isolated by F. Spindle from