Step (e). The small, chiefly microsomal, clearance pellets from the supernatants of steps b and c were combined and suspended in 10% sucrose plus 5% PVP (P_3, Table I). The centrifugal procedure employed here yields a mitochondrial fraction (P_2 washed twice) nearly free of microsomes (microsomal contamination is quickly evidenced by precipitation following cautious acidification of the suspension under phase microscopy), small pellets of nearly pure microsomal material (P_3), but leaves the mitochondrial wash supernatants ($P_2SN_{1 \text{ and } 2}$), and the original supernatant (SN), heavily contaminated with microsomes. The microsomes can be sedimented in sucrose-PVP by centrifugation at a R.C.F. of ca. 100000 x g (Spinco centrifuge) or by addition of $MgCl_2 \cdot 6H_2O$ (1 to 2 mg per ml) and spinning at ca. 25000 x g. This latter treatment promotes agglutination of microsomes without precipitating solubilized enzymes as *e.g.*, those of the glycolytic system.

The distribution of cytochrome oxidase activities in the various fractions as determined by Warburg manometry (Table I) were as expected from the microscopic determinations. The removal of microsomal material during washing of the mitochondrial pellet (P_2 unwashed) was evidenced microscopically by inducing microsomal precipitation in the wash supernatants ($P_2SN_{1 \text{ and } 2}$), and by determining the glycolytic activity of these fractions in the presence of the original supernatant(5). It may be of interest to note that rabies virus in subcellular fractions obtained by sucrose-PVP fractionation of infected mouse brains was not measurably reduced in infectivity by the presence of PVP(6). A modification of the methods reported here has been used to study intracellular distribution of catalase(7).

Summary. Addition of polyvinylpyrrolidone (PVP) to sucrose solutions used in extraction of subcellular components improved centrifugal resolution of particulates, helped to preserve morphologic integrity of subcellular elements, and aided in preservation of enzymic activities. PVP was particularly useful in counteracting irreversible agglutination of microsomes.

1. Granick, S., *Am. J. Bot.*, 1938, v25, 558.

2. du Buy, H. G., and Woods, M. W., *Phytopath.*, 1943, v33, 766.

3. Hogeboom, G. H., Schneider, W. C., and Pallade, G., *J. Biol. Chem.*, 1948, v172, 619.

4. du Buy, H. G., Woods, M. W., Burk, D., and Lackey, M. C., *J. Nat. Cancer Inst.*, 1949, v9, 325.

5. Woods, M. W., Hunter, J., and Burk, D., *Fed. Proc.*, 1954, 1073.

6. Habel, K., du Buy, H. G., Woods, M. W., and McKinstry, P. W., *Bact. Proc.*, 1952, 85.

7. Greenfield, R. E., and Price, V. E., Abstr. Amer. Chem. Soc. New York, N. Y., Sept. 1954, pg. 48C.

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W - V Variation Induced in *S. typhi, S. paratyphi C* and *Paracolobactrum ballerup.* (21289)

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The Vi antigen occurs in a number of Salmonellae and other Enterobacteriaceae. Colonies with this antigen are designated as V colonies, morphologically and serologically different from W forms which lack the Vi antigen and react only with 0 sera. A change of V cultures to W, called V-W variation, is commonly observed in the laboratory. Little is known, however, about the reverse process, *i.e.*, W-V variation. It does not occur spontaneously. Booy and Wolff(1) succeeded in inducing Vi antigen in *S. typhi* W by growing it in media containing live or killed *S. typhi* V (vi) and *Ballerup* Vi organisms. Working with *Escherichia coli,* an organism related to Salmonellae, Boivin *et al.*(2) showed that transformation experiments with this microbe are successful only when unstable but smooth

strains are used. This group of workers believed that the active principle involved herein is a nucleic acid or a complex containing it (3). Braun and Whallon(4) showed that in the presence of whole organisms and cell-extracts, especially of heterologous desoxyribosenucleic acid, mutants of Brucella and *E. coli* develop that ordinarily do not establish themselves.

These observations warranted two questions. First, if only *S. typhi* and *Ballerup* organisms containing a closely related Vi antigen can give impetus to the recovery of V forms from W cultures or if this can be accomplished also by adding *S. paratyphi C* with Vi antigen. Secondly, what is the role of the different fractions of Vi antigenic material in the induced W-V variation. Since desoxyribosenucleic acid (DNA) and ribosenucleic acid (RNA) seem to be of paramount importance for Enterobacteriaceae, experiments with these materials were also indicated.

Materials. Stock cultures of V strains, *S. typhi Bhatnagar I* Vi, *S. typhi 2 Vi, S. paratyphi C* Vi and *Paracolobactrum Ballerup* 1 Vi were maintained on egg slants. Subcultures on agar plates were made biweekly, and at least 1000 colonies checked from each transplant for V and W characteristics. Stock cultures of W strains, *S. typhi* 158 O, *S. typhi* 212 O, *S. typhi* H 901, *S. paratyphi* C O and *Paracolobactrum Ballerup* 1 O, as well as rough strains, *S. typhi* 58 R and *S. paratyphi C* R, were maintained on nutrient agar slants and checked for colony characteristics biweekly. For the experiments proper, stock cultures of known purity were inoculated into Tryptose (Difco, Inc.) broth, containing 1% of this peptone, and 0.5% sodium chloride, pH 7.2 Amounts of 10^{-2} ml were streaked from this medium, 20 to 22 hours after inoculation, to plates made of 1% Tryptose, 0.5% sodium chloride and 1.5% agar, pH 7.2, and the colonies observed morphologically as well as tested serologically with the aid of the slide agglutination method. Only those giving at least a 3+ reaction with Vi sera and negative with O sera were considered V colonies. Preliminary experiments showed that the most favorable incubation time for the plates was 18 to 20 hours. Heat-killed organisms, acetone precipitates, acid hydrolysates, DNA and RNA extracts were added to the Tryptose broth tubes. They were prepared according to the following procedures: The strains were grown in Tryptose broth for 20 to 22 hours, then centrifuged and the supernatant discarded. Heat-killed bacteria were prepared by suspending the organisms in 0.9 per cent sodium chloride to make a concentration of 10^7 microbes per ml saline, and keeping this suspension at 56°C in the water bath for one hour. Acetone-precipitates and saline extracts therefrom were produced according to the method of Webster *et al.*(5). The same outline was followed in making acetic acid hydrolysates. The starting material was growth in Tryptose broth collected by centrifuging. DNA and RNA extracts of the organisms were prepared by the procedure of Morse and Carter(6). Heat-killed organisms and bacterial extracts were used as soon as possible after preparation. They were added to the tubes of Tryptose broth just prior to inoculation, in concentrations equivalent to 10^6 organisms per ml of the medium. After culturing the respective strain in Tryptose broth containing heat-killed organisms or an extract for 20 to 22 hours, $5x10^{-3}$% of the growth was transferred to a Tryptose broth tube with the same heat-killed organisms or with the same extract. This procedure was repeated for 14 days, streaking check-plates before each transfer.

Results. Neither heat-killed organisms nor extracts from V, W and R cultures lead to the appearance of Vi antigen in rough strains *S. typhi* 58 R, *S. paratyphi* C, R). None of the W strains formed V colonies when cultured without the addition of heat-killed organisms or their extracts in 14 successive transfers.

The relationship of V to W colonies varied but remained equal to or higher than 910 to 90 in all experiments in which heat-killed V, W or R strains were added to V strains.

None of the W strains formed V colonies when W or R cultures or extracts were added to the medium.

Acetic acid hydrolysates of V organisms were ineffective.

Heat-killed V organisms, their acetone precipitates and DNA extracts, as well as in some cases also their RNA extracts, induced the ap-

TABLE I. Induced W–V Variation : No. of Subculture in Which V Colonies Were First Demonstrated.

W strain	W organism grown in presence of: *S. typhi* Bhatnagar I				*S. typhi* 2 Vi				*S. paratyphi* C Vi				*Paracolobactrum Ballerup* 1 Vi			
	W	A	D	R	W	A	D	R	W	A	D	R	W	A	D	R
S. typhi 158 O	2	4	4	7	3	1	2	0*	6	9	6	0	5	8	8	0
" 212 O	1	5	2	0	5	10	4	0	7	8	7	0	3	8	9	0
" 901 H	3	7	3	0	2	10	4	0	5	9	6	0	4	10	8	0
S. paratyphi C O	3	7	5	8	6	9	8	0	3	6	3	9	6	7	4	10
Pa. Ballerup 1 O	5	10	7	0	9	0	8	0	6	10	9	0	3	5	5	0

* V colonies not observed in 14 serial transplants.
W, whole organism, heat-killed; A, saline extract of acetone precipitate; D, DNA; R, RNA.

pearance of V colonies on plates streaked from Tryptose broth to which these materials were added, usually after several transfers. Table I shows the number of transfers in which this W-V change occurred.

Discussion. It was shown that W-V variation can be induced in susceptible Enterobacteriaceae by growing their W cultures in serial transfers in media containing heat-killed V organisms or certain extracts thereof. Heterologous cultures had a slower action than homologous microbes and material prepared from them. Only true W, *i.e.*, smooth, organisms underwent this change. The concept of the formation of individual Salmonella serotypes by loss variation is well known. According to this doctrine one has to suppose that all *S. typhi, S. paratyphi C,* as well as *Paracolobactrum Ballerup* 1 and 2 once harbored the Vi antigen. A reversal of the loss variation which led to the formation of pure W strains may be considered.

It is not possible, without testing enormous numbers of colonies, to be certain that Vi organisms are not present in small proportions avoiding detection when only a few thousand colonies are examined. In this case the addition of whole organisms or their fractions may supply materials favoring the multiplication of spontaneously occurring Vi forms, to such an extent that they establish themselves in competition to the W cells and become serologically demonstrable.

Since the activity of the transforming principle of pneumococcal types can be recognized at dilutions 5×10^7, it is possible that the activity of RNA, which was observed only in a few instances, was due to the presence of traces of DNA.

These experiments also indicate the close relationship of the Vi antigen of *S. paratyphi C* to that of *S. typhi* and *Paracolobactrum Ballerup.*

Summary. Heat-killed Vi organisms, the saline extracts of their acetone precipitates and DNA fractions induced W-V variation in *S. typhi, S. paratyphi* C and *Paracolobactrum Ballerup* strains.

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1. Booy, H. L., and Wolff, H. L., *A. van Leeuwenhoek,* 1952, v18, 183.

2. Boivin, A., Delaunay, A., Vendreley, R., and Lehoult, Y., *Experim.,* 1946, v2, 139.

3. ———, *Experim.,* 1945, v1, 334.

4. Braun, W., and Whallon, J., *Bact. Proc.,* 1954, v1, 42.

5. Webster, M. E., Landy, M., and Freeman, M. E, *J. Immunol.,* 1952, v69, 135.

6. Morse, M. L., and Carter, C. E., *J. Bact.,* 1949, v58, 317.

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