fibrin, while in others the fibrin was seen in the subsynovial tissues. Near the attachments to the bone, the injured capsule and ligaments showed similar changes consisting of oedema, fibroblastic proliferation and lymphoid-cell infiltration. The loose connective tissue beneath the synovial lining of the tendon sheaths presented a picture essentially the same as that seen in the joint synovia.

At 2, and 3 weeks there were still definite signs of acute inflammation of the synovial tissue as evidenced by swelling and by leucocytic and lymphoid-cell infiltration. There was great increase of the fibroblasts in the subsynovial tissue. Hemosiderin pigment also was found in large amounts in the phagocytic cells of the inflamed area. At this stage hyalinized masses of fibrin were seen within the synovial tissue and on the synovial surface. The tendon sheaths and the soft tissue surrounding the capsule and ligaments showed changes similar to those which were found after 1 week, except that there was more marked fibroblastic proliferation.

After 4 weeks, there was no external evidence of swelling or other signs of old injury. Microscopic examination of the tendon sheaths, synovial tissue, and ligaments still showed evidence of old hemorrhage. The number of fibroblasts was decreased, but the number of intercellular collagen fibers was increased. Many small capillaries were seen in this young connective tissue. In certain places the synovial membrane showed small foci of degeneration. There was still considerable oedema of the loose connective tissue but the infiltration with leukocytes and lymphoid cells was much less than that shown in the sections taken after 3 weeks.

Microscopic examination at 6 weeks showed complete healing of the traumatized parts. There was a late stage of fibrosis with shrinkage and contraction of the connective tissue structures.

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Phenolphthalein Starch Medium for Rapid Isolation of V. Cholerae.

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Among the various media that have been advocated for rapid isolation of *V. cholerae,* the most widely used is the alkaline peptone enrichment medium which, however, is by no means perfect. Dur-

ing a cholera epidemic in Peiping in the summer of 1932, we felt that a more efficient medium was desirable. To meet this demand the following medium (phenolphthalein-starch solution) was devised, utilizing the unique property of rapid fermentation of starch by *V. cholerae*[1] in a highly alkaline solution in combination with an indicator as an index for its growth. As the cholera epidemic was over when the technique of preparation of this medium had been perfected, its efficiency was tested out on stools seeded with *V. cholerae* and gave satisfactory results.

The medium is prepared by dissolving peptone (Witte) 2 gm., maltose 1 gm., potassium nitrate 0.5 gm., sodium carbonate (crystallized) 0.5 gm., sodium chloride 10 gm., and magnesium chloride (crystallized) 0.5 gm. in 900 cc. of distilled water. The solution is boiled for 3 minutes and filtered. 100 cc. of a 5% solution of soluble starch previously boiled for 2 minutes is added to 900 cc. of the above solution and mixed thoroughly. The mixture is filtered through cotton and then through asbestos filter, yielding a clear filtrate which is first measured and then sterilized by boiling for 3 minutes. (The solution cannot be autoclaved in the ordinary way as the starch is decomposed by prolonged heating). The sterilized filtrate is kept in ice chest. Just before use the reaction of the solution is adjusted to pH 9.0-9.2 in the following way: First 5 cc. of the indicator phenolphthalein (saturated solution in 50% alcohol) is added to each litre of the filtrate and the solution is carefully brought to decolorization point (pH 8.3) by addition of necessary amount of N/20 HCl or N/20 NaOH and finally to each litre of the decolorized solution is added exactly 1.5 cc. of N/I NaOH which brings the solution to pH 9.0-9.2 (pink colored). The titrated solution is distributed into separate sterile flasks in 50 cc. lots, ready for seeding. (The solution after titration for pH should be used fresh.)

For cultivation of *V. cholerae* from stool, 0.1-0.2 cc. of the liquid stool or a loopful of the solid stool is inoculated into 50 cc. of the P.S. medium (Phenolphthalein starch medium) and incubated at 37°C. for 5-8 hours. The decolorization of the solution which indicates growth of cholera or cholera-like vibrio is watched. The microscopic or macroscopic agglutination test can then be made from the surface growth of the decolorized solution with known anti-cholera serum. If decolorization is incomplete by the end of 8 hours' incubation or the growth is too thin for agglutination tests,

[1] Gordon, M. H., *Centralb. f. Bakt.*, 1906, **1**, 42.

1 cc. from the seeded P.S. medium is transferred to a fresh flask of P. S. medium and incubated for another 5-8 hours.

As a preliminary test for the efficiency of the P.S. medium a comparison of this medium with the A.P. medium (alkaline peptone enrichment medium[2]) is made. From each of the 30 specimens of non-choleraic stools 2 stool emulsions are made, (A) containing 2 loopfuls of stool and 0.5 cc. 18 hours' peptone water culture of *V. cholerae* in 5 cc. saline, and (B) with the addition of 0.5 cc. cholera-phage. From the individual stool emulsions, 0.2 cc. is inoculated separately into 50 cc. lots of the P.S. medium and the A.P. medium for both series and incubated at 37°C. for 6 hours. At the end of this period plating on C.R. medium (China-blue-rosalic-acid agar pH 7.6) and macroscopic agglutination tests from each flask of the P.S. and A.P. media are made.

1. *Agglutination tests.* From 30 specimens of the treated stools, the P.S. medium gave positive agglutination test for *V. cholerae* in 30 specimens or 100% in A. series and in 10 specimens or 33.3% in B. series; while the A.P. medium gave 20 specimens or 66.7% in A. series and 1 specimen or 3.3% in B. series. Thus, the P.S. medium gave a higher percentage of positive agglutination tests than that obtained from the A.P. medium whether cholera-phage was present or not. It is also shown that the presence of cholera-phage in stools containing *V. cholerae*, greatly reduces the percentage of positive isolations.

2. *Plate growths.* The percentage of specimens giving growth of *V. cholerae* and *B. coli* in C.R. plate from the A.P. and P.S. media is given in Table I, which shows that *V. cholerae* grows more easily in the P.S. medium by the end of 6 hours' incubation, and *B. coli* is somewhat inhibited in the P.S. medium.

TABLE I.

% of Positive Plate Isolation of *V. cholerae* from Alkaline Peptone and Phenolphthalein Starch Media.

Types of Colonies	A. Series Stool + *V. cholerae*		B. Series Stool + *V. cholerae* + choleraphage	
	Alkaline peptone	Phenolph. starch	Alkaline peptone	Phenolphthalein starch
	%	%	%	%
V. cholera	60	86.7	13.3	30
B. coli	96.7	66.7	96.7	73

The above results suggest that for the rapid isolation and diagnosis of *V. cholerae* from stools, the phenolphthalein starch medium

[2] Bengstone, I. A., *Hyg. Lab. Bull.*, 1924, **139**, 37.

is more satisfactory than the alkaline peptone enrichment medium, even in the presence of cholera-phage. Hence in convalescent cholera cases when cholera-phage has appeared in the stool, the use of phenolphthalein starch medium would be a distinct advantage. Furthermore, the presence of the indicator serves not only for titration of the medium to a desired alkalinity but also as a growth index of the culture. Although its value must not be over-estimated until data from clinical cases of cholera are available, it seems worth while to record here the favorable results obtained under experimental conditions with the phenolphthalein starch medium and to recommend its trial in cholera cases.

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Rough Forms of Vibrio Cholerae from Convalescents.

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It has been observed[1] that dissociation of *Vibrio cholerae* could be enforced by bacteriophage *in vitro* and the resulting variants showed morphological and serological changes from the parent strain. Pasricha and his coworkers[2] reported that by subjecting a freshly-isolated phage-resistant cholera strain to repeated cultivation on laboratory media they could obtain certain colonies showing morphological, cultural and serological variation. They also produced evidence suggesting that the loss of agglutinability induced by a high titered cholera serum might occur in cholera vibrios normally during the course of the disease.

During the summer of 1932 a fairly widespread epidemic of cholera extended to Peiping where a limited number of cases not treated with bacteriophage was available for study. The purpose of the present investigation was to make a careful study of culturally-positive cases from the beginning to the end of the disease so as to isolate rough forms of *Vibrio cholerae* and cholera bacteriophage from the stool and vomitus specimens obtained on different days of the disease. As soon as a specimen reached the laboratory

[1] Chen, W. K., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1160.

[2] Pasricha, C. L., de Monte, A. J., and Gupta, S. K., *Ind. Med. Gaz.*, 1932, **67**, 64.