earlier report[2] that milk products given in place of drinking water lead to an increased level of cholic acid in the gallbladder bile of the chick. In addition, it is shown that liquid buttermilk, whey and protein-free milk all exhibit an activity similar to that of skim-milk. The protein-free milk was made by isoelectric precipitation of casein, filtering, coagulation of remaining proteins by heating, filtering again and neutralizing. There remains no doubt that the greater part of the cholic acid-producing activity remains in milk after separation of the proteins of milk, and that the activity is resistant to heating to the boiling point at pH 4 to 5. Cholic acid-producing activity is not shown by the estrogen fraction from whey, or by the pure estrogens tested, or by another structurally related substance, cholesterol.

The results obtained in this study confirm previous reports of the existence in milk of a cholic acid-producing substance, possibly a precursor of bile acids, which is yet unidentified.

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Effects of Smallpox Vaccination (Vaccinia) on Serologic Tests for Syphilis.

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False positive serologic tests for syphilis have been attributed to many causes. More recently,[1,2,3,4] smallpox vaccination (vaccinia) has been added to the list. Lynch[3] and his associates, in a controlled study, showed that false positive reactions occurred in 16% of 263 vaccinated subjects. The Kolmer, Kline, Hinton and Mazzini tests were employed. One or more of these tests were positive, varying in intensity from ± to ++++. The reactions appeared in 2 weeks and remained positive for 2 months in most instances, but a few persisted for 4 months and one for 5 months.

An opportunity for a similar study was presented to us recently.[5] A group of individuals (mostly medical students and nurses) was chosen, in whom a serologic test had been performed within a reasonable time previous to the vaccination. There were 202 individuals, ranging in age from 3 weeks to 84 years. Over one-half were between 20 and 30 years of age. Forty-four individuals developed a primary cutaneous (non-immune) reaction at the site of inoculation, 134 developed a local accelerated (vaccinoid) form, and 24 were immune.

Out of 202 individuals on whom a single blood examination was performed, 19 were found to be positive, ranging in intensity from ± to ++++ by one or more of the Kolmer, Kahn and Mazzini tests. Not all persons were tested at the same time. They were examined from 10 days to 57 days following the vaccination, but 93.6% were done before the forty-third day. One month following vaccination 25 persons that gave a negative reaction were re-examined. Four out of this group were now positive. Undoubtedly, others would have been detected if the entire group had been examined more frequently.

In all, there were 24 individuals out of 202 who gave a false positive reaction for syphilis. This is an incidence of 11.8%. The majority of the reactions were ++ or less in intensity

1 Barnard, R. D., *Illinois M. J.*, 1940, **77**, 78.

2 Thomas, G. E., and Garrity, R. W., *U. S. Naval Med. Bull.*, 1941, **39**, 272.

3 Lynch, F. W., Boynton, R. E., and Kimbal, A. C., *J. A. M. A.*, 1941, **117**, 591.

4 Mohr, C. F., Moore, J. E., and Eagle, H., *Arch. Int. Med.*, 1941, **68**, 1161.

5 Favorite, G. O., *Phila. Med.*, in process of publication.

TABLE I.
Distribution and Degree of Intensity of False Positive Reactions for Syphilis Following Smallpox Vaccination.

Time following vaccination	14 days			30-43 days			60 days		
Patient's No.	Kolmer	Kahn	Mazz.	Kolmer	Kahn	Mazz.	Kolmer	Kahn	Mazz.
9	+	—	++	—	—	—	—	—	—
15	+	++	++	—	+++	+++	—	—	—
23	+	—	—				—	—	—
25	—	—	—	±	—	—	—	—	—
32	—	—	—	—	++	++	—	—	—
33	—	—	—	—	+	—	—	—	—
35	—	—	—	—	—	++	—	—	+
52	—	—	++				—	—	—
55	++	—	+				—	—	—
58	—	+	++				—	—	—
78				—	±	+++	—	—	—
84				—	—	+++	—	—	+
102	++++	++++	+++	++++	++++	+++	—	++	+++
103				—	+	±	—	—	—
110				—	+	±	—	±	±
118				+	—	±	+	—	+
135				—	+	+	—	±	+
139				—	—	±			
146				—	—	++	—	—	++
147				—	—	+	—	—	—
150				±	—	—	—	—	—
178				—	—	+	—	—	—
203				—	±	—	—	—	—
204	++++	++++		—	+	±	—	±	—

TABLE II.
Summary of Number and Degree of Intensity of Reactions Encountered in 202 Individuals Following Smallpox Vaccination.

Degree of reaction	±	+	++	+++	++++	Total
Kolmer	2	5	1	—	3	11
Kahn	5	6	3	1	3	18
Mazzini	6	9	8	6	—	29
Total	13	20	12	7	6	58

but 10 were +++ or stronger in one or more tests. The reactions were about equally divided between the non-immune and the accelerated (vaccinoid) groups. None occurred in the immune.

Table I shows the distribution and the degree of intensity of the reactions encountered. Those individuals that were positive at the end of 14 days following vaccination were still positive (with one exception) at the end of 30-43 days. Fifteen became negative in 60 days, while 8 still gave positive reactions but to a lesser degree.

Table II shows the number and the degree of reactions of the tests employed. Seventy-eight percent gave a reaction of ++ or less, while 22% were +++ or stronger.

Comment. To show that the incidence of false positive reactions in this study was not due to chance alone, a survey was made of the serologic tests performed previously in the same laboratory. Including all tests, without regard to their specificity, the incidence of positive reactions was 0.9% in 1000 Kolmer and 1000 Kahn premarital examinations. For the control series for the Mazzini test, 1000 consecutive blood donors from the general population were used. There were 2.4% positive tests. Employing this figure for calculating the significant difference, when compared to the experimental group of 11.8% in 202 individuals, the difference is highly significant. Furthermore, the reactions persisted in the same individuals and gradually disappeared spontaneously. The vaccination was the only apparent factor operating.

Summary. Evidence is presented in confirmation of the work of others that smallpox (vaccinia) vaccination gives rise to false positive reactions for syphilis. The incidence of positive reactions by the use of the Kolmer, Kahn and Mazzini tests was 11.8%. The false positive reactions are transitory but may persist for 2 months or more.

Addendum. The patients with positive reactions were retested every two weeks. There was a gradual diminution in the intensity of the reactions until all became negative by the end of 120 days.

I wish to express my gratefulness to Miss Helen Breen and Miss Kathryn Simmons for their technical assistance.

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Growth of *Penicillium Notatum* on Various Media and the Development of an Antibacterial Substance.

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The antibacterial action of penicillium was first observed by Fleming,[1] and a further report was made by Chain and others.[2] Subsequently the preparation and the properties of this substance were reported in greater detail.[3,4]

The more recent studies have been primarily concerned with the isolation of a substance designated as penicillin and the mold was grown on a modified Czapek-Dox synthetic medium.[3] It seemed desirable to make some observations with this organism on other media. Various procedures were used to increase the growth of the penicillium and the effect of such procedures upon the production of an inhibitory substance was determined.

The data reported in this paper include (1) the growth of the mold penicillium on 3 different media (modified Czapek-Dox, infusion broth, and Amigen); and the relation of the pH of the media to the production of an antibacterial substance; (2) the influence of the addition of various sugars on the production of bacterial inhibitory substance; (3) the extraction of an antibacterial substance from the thallus.

Materials and Methods. Culture Media: (1) Modified Czapek-Dox synthetic media; this will be referred to as the Oxford Media. (2) Difco stock heart infusion broth; (3) an enzymic digest of purified casein and pork pancreas in which the proteins have been hydrolyzed to amino acids 75% and di- and tri-peptides 25%. This preparation will subsequently be referred to by its trade name of Amigen.* The pH range of all media was 4.4 to 5.0 when inoculated. All pH determinations were made with a glass electrode.

Cultures: A strain of *Penicillium notatum* was obtained from Dr. N. F. Conant, Durham, N.C. Stock cultures were grown on Sabouraud's media. The cultures for study were all grown in 225 cc Ehrlenmyer flasks. A strain of hemolytic *Staphylococcus aureus* isolated from a patient with osteomyelitis was used as the test organism. Dr. Gladys Hobby kindly determined the sensitivity of this culture to several preparations of penicillin. It was found to be about one-fourth as sensitive to the drug as the test organism, a strain of hemolytic streptococcus (C 203 MV).[5] This

[1] Fleming, A., *Brit. J. Exp. Path.*, 1929, **10**, 226.

[2] Chain, E., Florey, H. W., Gardner, A. D., Heatley, N. G., Jennings, M. A., Orr-Ewing, J., and Sanders, A. G., *Lancet*, 1940, **2**, 226.

[3] Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W., *Lancet*, 1941, **2**, 177.

[4] Abraham, E. P., and Chain, E., *Brit. J. Exp. Path.*, 1942, **23**, 103.

* The media made with Amigen contained 10% of Amigen powder by weight dissolved in distilled water. The Amigen was supplied by Mead Johnson Co., Evansville, Indiana.

[5] Hobby, G. L., Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 277.