that our knowledge of the bacteriological conditions in the soil is still so limited that a general and successful inoculation with non-symbiotic nitrogen-fixing bacteria, with the decay bacteria, or with nitrifying bacteria is entirely out of the question for the present. The only direction in which soil inoculation has been rendered more or less practicable is the inoculation with cultures of various tubercle bacteria. Such inoculation can, therefore, be applied to legumes and *legumes* only. By so doing we may make possible the formation of tubercles and, therefore, the fixation of nitrogen by leguminous crops in such soils where the proper bacteria are naturally absent, but it should also be remembered that in most soils the failure of leguminous crops to grow satisfactorily is due not to the absence of the proper soil bacteria, but to general unfavorable soil conditions, to absence of a sufficient amount of lime, of a sufficient amount of humus, or of sufficient aeration. The inoculation of such soils without previous improvement would be a waste of effort and money. There is ample justification, therefore, to utter here a warning against misconception and unjustifiable expectation. Ignorance in this direction will be exploited, as ignorance in other directions has been exploited, by attempts to sell to farmers, improperly informed, cultures of soil bacteria advertised as the panacea for all soil-ills. Let the man who wishes to inoculate his soil remember that it is not yet practicable to inoculate it for wheat or potatoes, or melons and while it is practicable to inoculate it for alfalfa, or soy beans, or other legumes, he should inform himself as to the real facts before proceeding with his inoculation.

[CONTRIBUTION FROM THE FOOD DIVISION OF THE BUREAU OF CHEMISTRY, U. S. DEPT. OF AGRICULTURE. SENT BY H. W. WILEY.]

EXAMINATION OF LARD FROM COTTONSEED-MEAL-FED HOGS, BY PHYTOSTEROL ACETATE METHOD OF BÖMER.

BY L. M. TOLMAN.

Received March 1, 1905.

THE fact that lard prepared from hogs fed on cotton-seed meal will give positive tests with the Halphen and the Becchi reagents has been known for some time. Consequently, a question of

adulteration is always raised when these tests are found to be given by any sample of lard.

Recently, Fulmer[1] published the results of some careful and complete feeding experiments on hogs where varying amounts of cottonseed meal were fed, the object being to determine its effect on the lard, especially as regards the Halphen test for cottonseed oil, and the results show that little value can be attached to the test in an examination of lard, especially where cottonseed meal is likely to have been fed.

Through the kindness of Mr. Fulmer, fifteen samples of lard, prepared from the hogs used in his experiments, were obtained for this work. In his paper he has carefully described these samples, in detail, so that only a brief description of them will be given in Table II. Solstein[2] has called attention to some samples of lard, which he obtained in Chicago, and which gave a strong Halphen test, but did not show the presence of phytosterol,and he concluded that this test was final.

The lards obtained from Fulmer were prepared from different parts of the body of the animal, and gave reactions with the Halphen reagent, indicating from 0.5 per cent. to 15 per cent. of cottonseed oil. The unsaponifiable residue was examined microscopically, and the acetate prepared, and its melting-point determined.

The method used for estimation of cholesterol was that suggested by Böiner,[3] and is as follows: Saponify 100 grams of the fat with 200 cc. alcoholic potash (200 grams KOH to 1 liter of 70 volume per cent. alcohol), using a reflux condenser. Transfer the soap solution, while still warm, to a separatory funnel from 1.5 to 2 liters in capacity, and add 400 cc. of water. After cooling, add 800-1000 cc. of ether, and shake vigorousy for from two to three minutes. Then separate the ether layer, and transfer to a flask and distil the ether. The extraction of the soap solution is repeated four times with 200 cc. of ether, and the ether residues all united. These are treated with 20 cc. of the alcoholic potassium hydroxide solution, and heated for a few minutes; 40 cc. of water added, and extracted with ether. The ether solution is washed two or three times with 10 cc. of water, or until free from soap, then transferred to a crystallizing dish, and the ether allowed to

[1] This Journal, **26**, 837 (1904).
[2] *Z. f. Offent. Chem.*, **7**, 140-143, 1901.
[3] *Z. f. Nahr. u. Genuss*, **1**, 38, 1898.

evaporate spontaneously. The residue is dried, and then taken up with 95 volume per cent. alcohol, and allowed to crystallize slowly.

Cholesterol, under these conditions, forms a layer of crystals on the surface of the liquid, while phytosterol crystallizes throughout the solution. This difference in manner of crystallization is quite marked. Under the microscope, these two substances show their characteristic crystals, as seen in the accompanying plates.

After the crystals have been examined, the alcohol is evaporated, and the residue treated with acetic anhydride, as described by Bömer[1] in his phytosterol acetate test, as follows:

Add 2 to 3 cc. of acetic anhydride, heat to boiling, and evaporate off the excess of anhydride on a steam-bath. The residue is recrystallized from 95 per cent. alcohol several times, and the melting-point determined. Bömer claims to detect by this method 1 per cent. of added cottonseed oil. The author was able to detect 2 per cent. quite readily.

Before examining these lards, considerable work had been done with pure lard and beef tallow, and mixtures of these with cottonseed oil. In some of these mixtures, heated cottonseed oil was used. This oil had been heated for twenty to thirty minutes, at 260° C., until it no longer reacted with the Halphen reagent, but this heating had no effect on the phytosterol acetate test.

It was found that from 6 to 8 per cent. of cottonseed oil added to either lard or tallow affected the crystalline form of the cholesterol to such an extent as to be easily recognized, the peculiar telescopic crystals of mixtures of cholesterol and phytosterol being formed. With additions of 5 per cent., the telescopic crystals are not formed, but there is a decided change in the crystalline form, which is readily recognized. With less than 5 per cent. of added cottonseed oil, there is not enough change from the pure cholesterol crystals to be sure of detecting the mixture.

The phytosterol acetate test gives a means of detecting these smaller additions, although mixtures, ordinarily made, could be detected by a microscopic examination.

The addition of 5 per cent. of cottonseed oil to a lard raises the melting-point of the acetate from 114° (uncor.), which was the maximum obtained with pure lards, tallows, and with cholesterol prepared from gall-stones, to from 118° to 119° (uncor.), while

[1] *Z. f. Nahr. u. Genuss.*, **4**, 1070 (1901).

an addition of 2 per cent. of cottonseed oil was readily detected, as it gave a melting-point of 115.8° (uncor.).

The thermometer used in this work was graduated from 85° to 200°, and in making the determination of melting-point, was immersed in the glycerin bath up to 100° mark, so that only from 13° to 20° of the mercury column was out of the bath, and as this would make only a slight stem correction, no correction has been made in the results reported in this paper. The actual melting-points obtained are slightly lower than those reported by Bömer, but as the results are very uniformly lower than his, it is probably due to a difference in the end-point taken, and also to the stem correction which he applies.

The melting-point of the third crystallization was taken, for as a rule there was not enough to make a fourth crystallization.

In Table I are given the results obtained on a number of samples of known composition.

TABLE I.—PHYTOSTEROL EXAMINATION OF LARDS AND MIXTURES.

Description.	Manner of formation of crystals.	Microscopical appearance.	Melting-point. Third crystallization
Pure lard	Cholesterol.	Cholesterol.	113.0° C.
Pure lard	"	"	113.0° C.
Pure lard	"	"	114.0° C.
Cholesterol from gall stones	"	"	113.8° C.
Cholesterol from tallow	"	"	113.0° C.
Pure lard + 2 per cent. cottonseed oil	"	"	114.8° C.
Pure lard + 2 per cent. cottonseed oil	"	"	115.8° C.
Pure lard + 4 per cent. cottonseed oil	like mixed crystals	like slightly impure cholesterol	117.0° C.
Pure lard + 5 per cent. cottonseed oil	mixed crystals	mixed	118.2° C.
Pure lard + 5 per cent. cottonseed oil	mixed crystals	mixed	119.4° C.
Pure lard + 5 per cent. cottonseed oil	mixed crystals	mixed	119.6° C.
Pure lard + 5 per cent. cottonseed oil	mixed crystals	mixed	118.° C.
Pure lard + 8 per cent. cottonseed oil	like phytosterol	telescopic crystals	119.5° C.

From these results it may be seen that very small additions of cottonseed oil can be detected in this way.

In Table II are given the results obtained on 15 samples of lard obtained from Fulmer, all of which gave the Halphen test.

TABLE II.—PHYTOSTEROL EXAMINATION OF LARDS FROM COTTONSEED-MEAL-FED HOGS.

Food laboratory No.	Elton Fulmer's No.	Source of fat.	No. of animals in lot.	Total amount of cottonseed-meal eaten. Pounds.	Pounds of meal eaten per 100 pounds. Pounds.	Coloration with Halphen reagent. Per cent. cotton-seed oil.	Crystal formation.	Crystalline form.	Melting-point of third crystallization of the acetate. °C.
11161	1214	Kidney	4	47.0	35.9	15.0	Cholesterol	Cholesterol	113.0
11171	1265	"	4	37.0	20.2	15.0	"	"	113.5
11164	1249	Back	3	48.0	24.0	11.0	"	"	Lost
11170	1257	"	6	27.3	34.1	9.0	"	"	112.0
11162	1242	Jowl	1	154.0	75.0	8.0	"	"	Lost
11165	1219	Kidney	5	98.0	44.8	8.0	"	"	114.0
11167	1194	"	6	91.0	65.5	8.0	"	"	113.4
11169	1191	"	6	27.3	34.1	7.5	"	"	113.0
11175	1200	Intestines	1	154.0	75.0	7.5	"	"	113.6
11166	1227	Jowl	2	115.0	53.5	5.5	"	"	Lost
11168	1268	Intestines	5	98.0	44.8	5.5	"	"	113.6
11173	1267	Back	4	16.6	7.3	4.0	"	"	113.5
11163	1413	Composite	8	40.0	44.0	3.0	"	"	Lost
11174	1221	Intestines	4	16.6	7.3	1.0	"	"	112.0
11172	1225	Back	1	7.7	1.8	0.6	"	"	113.8

The lards in Table II are arranged in the order of the amount of coloration given with Halphen reagent. This varies from No. 11161, which gives a coloration equal to 15 per cent. of cottonseed oil to No. 11172, which gives a coloration equal to 0.6 per cent. of cottonseed oil.

The formation of the crystals was, in every case, like those from pure lard and pure cholesterol. Microscopically, they all looked like pure cholesterol.

The melting-points of the acetate were in no case higher than those obtained on pure lard, that is, 114°, and in all but two, ranged between 113° and 114°. The lower melting-points obtained for two samples were undoubtedly due to slight impurities, which are hard to remove in such small quantities as are obtained.

The melting-point of the acetate obtained from sample No. 11161, which gave a Halphen test indicating 15 per cent. of cottonseed oil was 113.5°, while that of No. 11172, which gave a test indicating 0.6 per cent., was 113.8°, showing pure cholesterol in both cases. This method, therefore, will differentiate between lards which give the Halphen test, because of added cottonseed oil and lards which give the test because of the feeding.

The fact that cholesterol has always been found in lards, tallows and similar animal fats, is well known, and yet the animals from which these fats are derived are very largely fed on vegetable foods containing phytosterol, such as Indian corn, and linseed meal, and yet the phytosterol from these substances has never been found in the fat. By analogy, it would be expected that the phytosterol from cottonseed meal would not appear in the hog fat.

These considerations give added weight to the analytical results in Table II. The results of this work may be summed up in the following conclusions:

First, that lard from cottonseed-meal-fed hogs does not contain phytosterol, which confirms Solstein's work.

Second, that small amounts of added cottonseed oil can be detected in lard by this method, confirming Bömer's work.

Third, that heated cottonseed oil, which does not give the Halphen test, can be detected by this method.

Plate I shows the cholesterol crystals obtained from a sample of lard rendered in the laboratory.

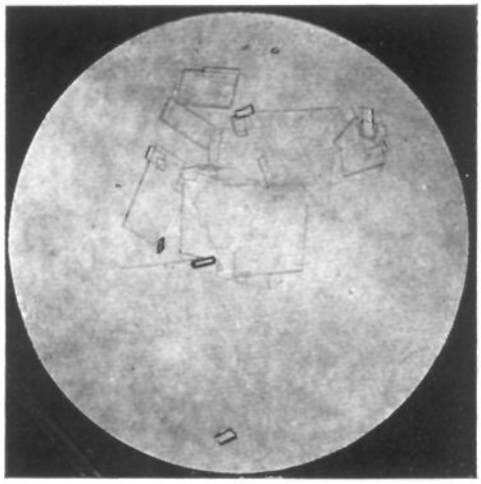

Plate I.

Plate II.

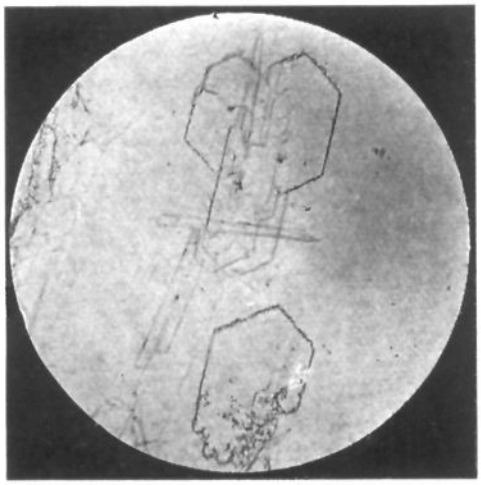

Plate III.

Plate IV.

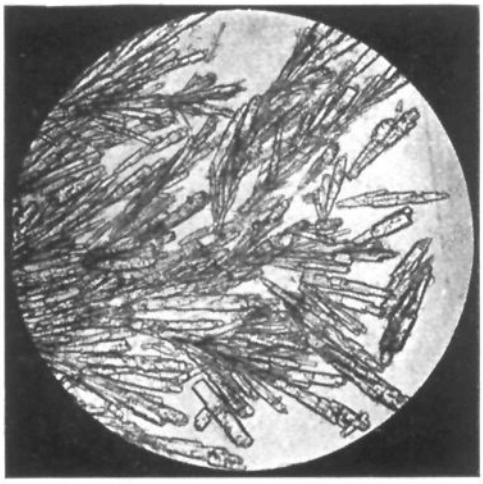

Plate V.

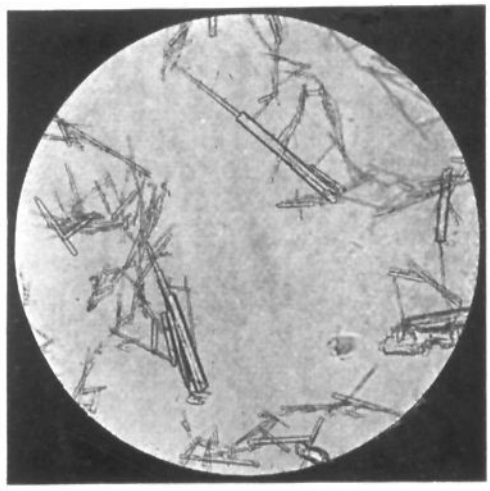

Plate VI.

Plate II shows the cholesterol crystals from one of Fulmer's lards, which gave a Halphen reaction about as strong as if 10 per cent. of cottonseed oil had been present.

Plate III shows phytosterol crystals from cottonseed oil.

Plate IV shows crystals from a mixture of 75 per cent. cholesterol, and 25 per cent. phytosterol with the characteristic telescopic effect produced by mixtures.

Plate V shows crystals from lard containing 10 per cent. of added cottonseed oil.

Plate VI shows crystals from lard containing 10 per cent. of heated cottonseed oil. This lard did not give the Halphen test, but the presence of cottonseed oil is at once detected in the form of the crystals.

The photomicrographs of the crystals were made by Mr. B. J. Howard, of the Bureau of Chemistry.

NOTE.—After this article had been submitted for publication, another paper on the same subject, by Emmett and Grindley, appeared in this Journal.[1] Their conclusions being so entirely opposed to those of Bömer, Solstein and others, as well as to the results of this paper, the author has felt compelled to call attention to what appears to him the weakness in their proof. They state that the crystals of the unsaponifiable residue, which they obtained from the lard from hogs fed on cottonseed oil appeared like phytosterol. Granting this to be true, there must have been a very large percentage of the phytosterol present, for as will be seen from Plate IV, 25 per cent. of phytosterol and 75 per cent. of cholesterol still give the peculiar telescopic form of crystal. In fact, the author has found that before crystals are obtained, which cannot be distinguished from phytosterol, at least 50 per cent. of phytosterol must be present. With such an amount, as must have been present in order to give the phytosterol-shaped crystals, it would be extremely easy to detect its presence by the melting-point of the acetate, which would be about 123 to 125° C., instead of 114° C., the melting-point of pure cholesterol acetate. This, the authors say they were unable to do.

The author working on a large number of lards from all parts of the body, some of which gave very marked reactions with the

[1] **27**, 263 (1905).

Halphen test, was unable to detect phytosterol, although the presence of phytosterol was easily recognized when only 2 to 3 per cent. of cottonseed oil was added. The crystals from these lards were all true cholesterol forms, and gave the correct melting-point when treated with acetic anhydride and purified.

[CONTRIBUTION FROM THE HAVEMEYER LABORATORIES, COLUMBIA UNIVERSITY, NO. 104.]

A STUDY OF METHODS FOR THE DETERMINATION OF FORMALDEHYDE.

BY R. H. WILLIAMS.

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THE work herein described consists of a comparison and criticism of the four methods most generally recommended for the determination of formaldehyde—Legler method,[1] hydrogen peroxide method,[2] iodimetric method,[3] and potassium cyanide method.[4] Having no means of obtaining formaldehyde in a pure, anhydrous state it became necessary to study the accuracy of these methods by indirect and comparative, rather than by direct and absolute tests. First, the conditions usually given for these methods were followed, and then such modifications tried as were believed to give increased accuracy or greater rapidity, the ultimate object being to determine, if possible, the best conditions for each method, and then to form a judgment as to their comparative merit.

Legler Method.—The conditions maintained were essentially those given by Smith.[5] Thirty determinations of formaldehyde were made under these conditions variously modified, and the results obtained established the following facts: That the best end point is obtained when a rather dilute solution of formaldehyde is taken, and N/5 reagents are used. That it is best to carry on the reaction in small flasks or bottles, thus minimizing the free space over the reacting liquids. That, after standing twenty-four hours, the maximum absorption takes place when the excess of ammonia is 1 cc. or more, and when the concentration of the

[1] Legler: *Ber. chem. Ges.*, **16**, 1333.
[2] Blank and Finkenbeiner: *Ibid.*, **31**, 2979.
[3] Romijn: *Z. anal. Chem.*, **36**, 18-24.
[4] *Ibid.*
[5] This Journal, **25**, 1028.