able to prepare a recovered product so that it would give the results obtained with Kahlbaum's chemically pure, dry carbon tetrachloride.

As a result of this work the following conclusions are offered:

(1) That much better results are obtained by the Wijs and Hanus solutions than by the Hübl.

(2) That the Hanus solution gives results much more closely agreeing with the existing data and is easier to prepare, but an excess of 60 to 70 per cent. is necessary to obtain quick action.

(3) That the Wijs solution is more rapid in its action, and an excess of 35 per cent. is sufficient, effecting a large saving in reagents and time of titrating, but it gives higher results.

(4) That thirty minutes is sufficient time for the action of either the Hanus or Wijs solution.

(5) That acetic acid is a better solvent for the work than carbon tetrachloride.

(6) That the bromine solution or iodine chloride or iodine bromide in carbon tetrachloride are not satisfactory for ordinary work.

(7) That iodine chloride in carbon tetrachloride is the most satisfactory solution, if determination of substitution is to be made.

(8) That both the iodine chloride and iodine bromide, being much less volatile than the bromine, there is much less danger of loss in that way with the former reagents.

The author wishes to acknowledge the assistance of Mr. Albert F. Seeker, of this laboratory, who repeated a large amount of the analytical work as a check on the results.

[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY, WASHINGTON AGRICULTURAL COLLEGE AND SCHOOL OF SCIENCE.]

ON THE REACTION OF LARD FROM COTTONSEED MEAL-FED HOGS, WITH HALPHEN'S REAGENT.

BY ELTON FULMER.

Received March 18, 1904.

IN December, 1902, we published[1] some results obtained by the application of Halphen's test to samples of lard rendered from the fat of hogs which had been fed with a ration containing

[1] This Journal, **24**, 1148-1155 (1902).

cottonseed meal. It was shown that lard obtained from the fat of two lots of hogs, six in each lot, which had received $66^{1}/_{6}$ pounds, and $135^{1}/_{12}$ pounds cottonseed meal, respectively, during a period of fifty-six days, gave a coloration with Halphen's reagent equivalent to that given by mixtures containing 2 and 4 per cent. of cottonseed oil. It was further shown that the lard obtained from the fat of two similar lots of hogs, which had eaten no cottonseed meal for a period of fifty-six days, but whose ration prior to the experiment had contained some of it, responded to the test with an intensity of color equivalent to that given by 1.2 per cent. and 2.8 per cent. cottonseed oil in admixture with normal lard.

It was stated at this time that a series of feeding experiments had been planned so that samples of lard might be obtained from the fat of hogs which had eaten varying amounts of cottonseed meal per day for the same period of time, and the same amount per day for different periods of time. These feeding experiments began January 29, 1903, and closed April 23, 1903, except in case of lot IV, with which the feeding of cottonseed meal continued until May 7, 1903. Twenty-three hogs were used, being divided for the purpose of the experiments into four lots. Lots I and II contained each six animals: lot III, eight; and lot IV, three.

RATIONS FED.

Lot I received ¼ pound cottonseed meal per day for each animal, with chopped wheat sufficient to make full feed.

Lot II was given ½ pound cottonseed meal per day for each animal, their grain ration being chopped barley.

An attempt was made with lot III to keep the amount of cottonseed meal equal to one-fifth of the grain ration, although it could not be kept up to this high proportion all the time. Chopped oats were fed until February 18th, and chopped barley for the remainder of the period.

The three animals in lot IV were fed all the time as much cottonseed meal as they could be forced to eat, with the view of killing them, if possible, by overfeeding. It will be noted in subsequent pages that the amounts actually eaten by them were very large, but their health was apparently unaffected. Their grain ration consisted entirely of chopped wheat.

All four lots were allowed freedom for exercise, received plenty of water, and were given an abundance of succulent food in the shape of carrots and sugar-beets.

One animal each from lots I, II and III, was killed at the end of the second, fourth, sixth, eighth, tenth and twelfth week. One of the two remaining in lot III was killed July 26th, three months after receiving the last ration of cottonseed meal, while the other was not killed until September 20th, five months after the close of the feeding period. Two animals of lot IV were killed at the end of fourteen weeks, the remaining one, which was in perfect health, being kept for farm stock.

When each animal was killed, four samples of fat were obtained by the writer, taken from the jowl, back, intestines and kidney, and in several cases a sample was also taken from the belly. Within a few hours after the fat was obtained, it was rendered in the laboratory, at low temperatures, in porcelain-lined kettles. Except in a very few instances, a sufficient amount of fat was used to yield at least one pound of lard. Each sample of lard thus obtained was treated with Halphen's reagent in the usual manner. In each test were employed 5 cc. lard, 5 cc. amyl alcohol, and 5 cc. carbon disulphide containing 1 per cent. of sulphur. Check samples were freely employed, using lard from hogs raised on the normal feed produced in this section. In no case was the slightest coloration observed with them.

For purposes of comparison, mixtures were made with check lard containing various known percentages of cottonseed oil. Preliminary tests were made to determine the approximate degree of coloration produced by Halphen's reagent with the several lards, and then those lying between certain limits were heated simultaneously in a bath of glycerol and water, together with known mixtures. For example, those samples showing a coloration equivalent to less than 2 per cent. cottonseed oil, were treated with Halphen's reagent, and then heated to 110° C. for fifteen minutes, side by side with mixtures containing 0.5, 1, 1.5 and 2 per cent. of the oil.

In this manner uniformity of conditions was insured. Similarly, all the samples showing in the preliminary test an equivalent greater than 2 per cent. cottonseed oil were treated in groups, with known mixtures in which the oil content differed increas-

ingly by 1 per cent. The heating of so many samples in open test-tubes was a disagreeable operation owing to the odors evolved. Hence we regard the suggestion of Steinmann[1] that the operation be carried out in sealed glass tubes, as a timely one. It is claimed that by this method the reaction takes place as quickly, and is as plain as when the test is conducted according to the original method.

The cottonseed oil used in preparing the known mixtures was "Prime Summer White." Its iodine value was 107.7. The results of these comparative tests, together with detailed information concerning each lard sample, are given in the following tables. For easy comparison we give tables showing results from animals from different lots which were fed varying amounts of cottonseed meal for the same period of time, and other tables representing each lot by itself, thus bringing together results from animals which had received very approximately the same amount of cottonseeed meal, per 100 pounds body weight, for varying periods of time.

Whatever the substance may be which is transmitted from the cottonseed oil into the animal fat, and to which the coloration with Halphen's reagent is due, it certainly is distributed to all parts of the animal, although in unequal amounts. In only one case did the samples of lard from all the different parts of the same animal show the same degree of coloration, and that was with animal No. 7 in lot III, which was killed July 26th, three months after it had received its last ration of cottonseed meal. The variations in coloration are not dependent upon the amount of meal eaten by the animal. For example, the extremes of coloration in case of animal No. 1 in lot I are 1 to 2½. This is the ratio also found with No. 2 of lot IV; yet the former had eaten only 7.7 pounds, while the latter had consumed 115 pounds of cottonseed meal.

With thirteen of the twenty-two animals killed, the kidney fat showed the greatest coloration, although that from the back, jowl and belly each equaled it in one instance. With two animals the fat from kidney, back and jowl gave the same degree of coloration. Four gave back fat which responded most strongly to the color reaction, while belly fat and intestinal fat gave the strongest color, each in one instance. With eleven animals, lard from intestinal fat gave the least coloration. In twelve cases jowl fat

[1] *Schweizer. Wochenschr. Chem. and Pharm.*, **39**, 360 (1901).

No. of lot.	No. of animal.	Days of feeding period.	Pounds of cottonseed meal eaten.	Pounds of cottonseed meal eaten per 100 pounds body weight.	Coloration equivalent to per cent. cottonseed oil in known mixtures.				
					Intestines.	Jowl.	Back.	Kidney.	Belly.
I	1	13	7.7	1.8	0.4	0.6	0.6	1.0	..
II	1	13	11.6	5.1	1.5	3.0	3.0	3.0	..
III	1	13	38.0	9.2	1.5	1.5	2.0	3.0	..
I	2	28	8.0	3.8	3.0	2.0	3.0	4.0	..
II	2	28	20.8	10.0	0.6	1.0	1.2	0.6	..
III	2	28	45.0	16.8	2.0	4.0	5.0	5.0	..
I	3	41	13.5	5.8	1.0	0.6	0.6[1]	1.5	..
II	3	41	30.2	15.7	1.5	4.0	3.0	4.0	..
III	3	41	48.0	24.0	8.0	4.5	11.0	5.5	..
I	4	55	16.6	7.3	1.0	2.0	4.0	2.2	4.0
II	4	55	37.0	20.2	4.0	4.0	5.0	15.0	5.5
III	4	50[2]	47.0	35.9	10.0	9.0	9.0	15.0	..
I	5	70	16.5	9.0	1.5	3.0	3.5	2.0	1.0
II	5	70	18.3	22.0	3.5	7.0	8.0	5.0	4.0
III	5	70	98.0	44.8	5.5	7.0	3.0	8.0	5.5
I	6	84	10.2	12.3	5.0	4.5	4.0	3.0	5.5
II	6	84	27.3	34.1	2.0	4.0	9.0	7.5	4.5
III	6	84	91.0	56.5	4.0	5.0	6.5	8.0	6.5
I	1	13	7.7	1.8	0.4	0.6	0.6	1.0	..
I	2	28	8.0	3.8	3.0	2.0	3.0	4.0	..
I	3	41	13.5	5.8	1.0	0.6	0.6	1.5	..
I	4	55	16.6	7.3	1.0	2.0	4.0	2.2	4.0
I	5	70	16.5	9.0	1.5	3.0	3.5	2.0	1.0
I	6	84	10.2	12.3	5.0	4.5	4.0	3.0	5.5
II	1	13	11.6	5.1	1.5	3.0	3.0	3.0	..
II	2	28	20.8	10.0	0.6	1.0	1.2	0.6	..
II	3	41	30.2	15.7	1.5	4.0	3.0	4.0	..
II	4	55	37.0	20.2	4.0	4.0	5.0	15.0	5.5
II	5	70	18.3	22.0	3.5	7.0	8.0	5.0	4.0
II	6	84	27.3	34.1	2.0	4.0	9.0	7.5	4.5
III	1	13	38.0	9.2	1.5	1.5	2.0	3.0	..
III	2	28	45.0	16.8	2.0	4.0	5.0	5.0	..
III	3	41	48.0	24.0	8.0	4.5	11.0	5.5	..
III	4	50[2]	47.0	35.9	10.0	9.0	9.0	15.0	..
III	5	70	98.0	44.8	5.5	7.0	3.0	8.0	5.5
III	6	84	91.0	56.5	4.0	5.0	6.5	8.0	6.5
III	7	84[3]	45.5	34.2	4.0	4.0	4.0	4.0	
III	8	84[4]	40.0	44.0	A composite sample = 3.0.				
IV	1	98	154.0	75.0	7.5	8.0	8.0	8.0	4.0
IV	2	98	115.0	53.5	8.0	5.5	9.0	13.0	13.0

[1] Rendered with skin.
[2] Died March 19th.
[3] Killed three months after receiving last ration of cottonseed meal.
[4] Killed five months after receiving last ration of cottonseed meal.

responded with less color intensity than back fat, and in five they were equal. It will be thus seen that the tendency seems to be for hogs to store up in their kidney fat the greatest amount of the color-producing substance, the amount stored in back fat being less, in jowl fat still less, and least in the intestinal fat. The case of animal No. 7 in lot III is an interesting one. It had received no cottonseed meal in its ration for three months prior to being killed, but the fat from all parts of its body gave the same degree of coloration, equivalent to 4 per cent. cottonseed oil. This is but a single instance, and it is therefore questionable whether this equalization in distribution of the color-producing principle has any significance; but the persistency of its presence in the body fat long after the animal has ceased to receive it in its ration is very full of significance.

It does not appear from these experiments that either age, breed or sex has any relation to the amount of the color-producing principle that is deposited, or to its distribution to different parts of the body.

It is the opinion of Dr. B. Sjollema and Dr. J. E. Tulleken[1] that the coloration obtained with Halphen's reagent should not be expressed in percentages because different cottonseed oils respond with different intensities of color. We only partially concur in this opinion. We have observed these differences in cottonseed oils, as shown in subsequent pages, and have further observed that, in a series of experiments such as we have carried out, the same oil when mixed in various proportions with normal lard will, occasionally, for reasons now unknown, give a marked yellowish red coloration with Halphen's reagent, which makes comparison exceedingly difficult. Furthermore, we have found that after the amount of cottonseed oil in known mixtures has reached 8 per cent. it becomes increasingly difficult to detect variations in intensity of color produced by the further addition of 1 or even 2 per cent.; and when 12 per cent. has been reached, the difficulty increases to almost an impossibility. Because of this fact, we regard the high percentages (above 12 per cent.), recorded in the preceding tables, as approximations, although probably not far from correct. No such trouble is experienced with low percentages, save when the standard mixtures assume a mixed color, as above indicated.

[1] Mittheilung aus der Landwirthschaftlichen Reich-versuch Station, Groningen, Holland.

As already stated, the results given in the foregoing tables were obtained by comparing the coloration produced by the various lards with Halphen's reagent, with the color given under the same conditions by mixtures containing known percentages of cottonseed oil—the oil used for the purpose being known as "Summer white." Mixtures were also made by the use of normal lard and "Winter yellow" oil, and these, as well as those containing "Summer white" oil were used for comparison with sixty-two out of the entire number of ninety-six samples of lard. In every instance the comparison with "Winter yellow" mixtures resulted in showing a lower percentage of color-producing substance in the lards under investigation. As one would expect, the difference was nearly a constant one throughout the entire series. For example, Sample No. 1189, which, when compared with the "Summer white" mixture, showed a coloration equivalent to 5 per cent. of cottonseed oil, gave a color equivalent to only 4 per cent. of oil when comparison was made with the "Winter yellow" mixtures. A few more examples of this fact are given below:

	Coloration equivalent to per cent. cottonseed oil when compared with	
Laboratory number.	"Summer white" mixtures.	"Winter yellow" mixtures.
1189	5.0	4.0
1184	3.0	2.5
1185	6.5	5.5
1193	4.0	3.0
1230	4.0	3.0

These results simply indicate that the color-producing substance is present in the "Winter yellow" oil in greater amount than in the "Summer white," or, in other words, a given intensity of color is produced by a smaller percentage of the former than of the latter. In the comparative tests made, an approximate difference of 20 per cent. was observed in nearly all cases Because of these facts the objection to expressing the degree of coloration obtained in any particular test, in terms of percentage of cottonseed oil, is doubtless well grounded, if the case is one demanding extreme accuracy. It is doubtful, however, if the differences in cottonseed oils are sufficiently great in this respect to make necessary a discontinuance of the practice of thus expressing results obtained by the application of Halphen's reagent,

for extreme accuracy is not essential to credibility in work of this character inasmuch as all chemists recognize the limitations of color reactions. To obtain results from unknown conditions that are comparable with those from known ones is, after all, the purpose of this reagent, and this purpose is certainly accomplished even though the degree of coloration is not constant for all cottonseed oils. It is obvious, however, that when results are expressed in percentages, a statement should be made as to the kind of cottonseed oil used in the comparative determinations.

Our work has corroborated the statement of Swaving[1] that the intensity of the color increases shortly after cooling. This change is so marked that if the standard samples are kept warm, and those being tested are allowed to become cold, they will uniformly show a higher percentage equivalent of color than if comparison is made while they are still hot. For this reason also, if results obtained by use of Halphen's reagent are to be expressed in percentages, there should be a statement of the conditions under which the comparison is made. In our own work it has always been made when both the standard samples and those under investigation were hot.

Although there is this change in intensity of color upon cooling, the final color reaction is a permanent one if kept in the dark, as Swaving also observes. We have in our laboratory samples of cottonseed oil, and also of lard containing it, to which Halphen's reagent was added more than two years ago. These have been kept in the dark a portion of the time, and a part of the time have been exposed to diffused daylight; but the intensity of color is apparently the same now as when the samples were first prepared.

Swaving[1] has found that when cows are fed upon cottonseed meal the butter produced from their milk will give the color reaction with Halphen's reagent. This is not a new observation so far as the reaction for cottonseed oil in butter is concerned, whether obtained by Halphen's reagent or by other means, but we refer to it here because of the interesting details that are published relative to it. When two kilos cottonseed meal per day were fed for two days the butter gave a coloration equivalent to 0.5 per cent. cottonseed oil, and the same amount per day fed for eight days resulted in a coloration given by the butter equivalent

[1] *Ztschr. für Nahrungs und Genus.* **3**, 97-107 (1903).

to 2 per cent. Six days after the feeding of the meal stopped, the butter gave no reaction. When the ration of meal per day was increased to 4 kilos the butter gave colorations equivalent to percentages of oil as follows: After four days, 4 per cent.; seven days, 4 per cent.; eleven days, 5 per cent.; eighteen days, 4 per cent.; one day after meal ration stopped, 4 per cent. It is thus seen that the color makes its appearance very quickly in the butter fat (Swaving says within twenty-four hours after the meal is fed) and gradually increases with the amount of meal used in the ration, and that when the feeding ceases the reaction diminishes and quickly disappears—a reaction equivalent to 2 per cent. of cottonseed oil disappearing in six days. These facts, of course, relate to the fat normally secreted by the cow. It would have been interesting to know if the body fat of the cow producing the butter would also have responded to Halphen's reagent. There is certainly a marked difference between butter and lard as to the persistency of the color reaction after it once makes its appearance in the fat. In case of the butter just mentioned, the reaction very quickly vanished, while it will have been noted in the preceding tables that a composite sample of lard from the animal killed September 20th, or five months after the close of the feeding period, still gave an intensity of color equivalent to 3 per cent. cottonseed oil. The cow is concerned with the production of two kinds of fat, *viz.*, butter fat and body fat, while with the hog body fat is chiefly produced. The color-producing principle appears in the butter fat twenty-four hours after cottonseed meal has been fed; and, although it persists but six days after the meal has been withdrawn from the ration, yet we must ask from whence is it obtained during these six days. Does it remain in the circulatory system all the time, or is it first deposited in some of the tissues, and later reenter the circulation? It is known that the body fat of cottonseed meal-fed cattle will respond to Becchi's test; and it is not difficult, therefore, to see in these facts a possible relation to the much-discussed problem as to whether or not a cow normally draws upon her body fat in the production of butter fat. Unless this active principle of cottonseed oil remains for six days in the circulatory system, which is not probable, it must be deposited somewhere; it is well known that it may be deposited in the body fat; and, inasmuch as butter

continues to respond to its presence for six days after the last ration containing it has been eaten, no violence is done to logic in concluding that at least some constituents of the body fat are reabsorbed and make their appearance in the butter fat. There is nothing to indicate that the experimental cows of Swaving were forced to draw upon stored-up materials for lack of proper food, but rather, we are led to believe, that the conditions after the feeding experiment closed were normal for milk production.

From the evidence that follows it appears that the hog probably does not store up the cottonseed oil in its fat unchanged, although this is doubtless a possibility, as Munk[1] (and others) has shown by a very interesting experiment. A dog was starved until it had been deprived of its fat, as shown by the sudden increase in the amount of nitrogen excreted. The animal was then fed on colza oil and killed. The fat found in the various organs was more liquid than ordinary dog fat, and analysis showed it to contain 82.4 per cent. of oleïc acid, whereas normal dog's fat contains only 65.8 per cent. of this acid. Furthermore, the presence of erucic acid, which is never a normal constituent of animal fat, but which does occur in colza oil, was demonstrated.

Lebedeff[2] had previously carried out similar experiments with dogs, feeding linseed oil and mutton fat, the former causing the animal fat to have a very low point of congelation, while the latter caused it to have a high melting-point. These experiments seem clearly to prove that fats and oils may be absorbed and deposited unchanged. Yet the experimental evidence at hand seems, to us, to point to a negation of this possibility as relating to the deposition of cottonseed oil unchanged in the fat of hogs.

Soltsien[3] maintains that Halphen's test cannot be used to determine whether cottonseed oil has been added to lard (because of the reaction given with lards from hogs fed on cottonseed meal), but that the presence of phytosterin is conclusive of such adulteration. Acceptance of this idea naturally forces the conclusion that the color-producing substance in the oil is deposited unchanged in the animal fat, while another normal constituent of the oil, phytosterin, is not thus deposited. The only evidence he gives on this point is the fact that he obtained from American lard, made from the fat of hogs that had been fed on cottonseed

[1] J. Munk : *Virchow's Arch.*, **95**, 407 (1884).
[2] A. Lebedeff : *Med. Centrlbl.*, **8** (1882).
[3] *Ztschr. öffentl. Chem.*, **7**, 140-143 (1901).

meal, a reaction with Halphen's reagent, but failed to detect phytosterin.

As above stated, the experiments of Munk and Lebedeff demonstrated that the nature of the fat fed influenced, in large degree, the body fat of the animal, the feeding of colza oil serving to increase the oleïc acid content of dog fat about 25 per cent. Why should we not expect that similar results would be obtained in experiments with other animals? And that a liberal allowance of cottonseed meal for a number of weeks would furnish an amount of oil which, if stored in the fat, would be sufficient to greatly modify its characteristics? A number of experimenters have shown that such is the case. For example, as long ago as 1893 Harrington and Adriance[1] observed that a ration of cottonseed meal, modified to a greater or less extent, the body fat of hogs, sheep and cattle, and also the butter fat of milch cows. At this time Halphen's test had not been published, and the presence of cottonseed oil in fats was demonstrated by means of Becchi's reagent. They found that the melting-point of butter produced from cows that had been fed on cottonseed meal increased steadily with the amount of meal fed, and that generally the iodine number was also increased. In every case such butter strongly reduced Becchi's reagent. They further found that the lard from cottonseed meal-fed hogs reduced silver nitrate, and had a higher melting-point and lower iodine number than lard from corn-fed hogs. In case of sheep, the mutton fat had a higher melting-point and a considerably higher iodine value than that from sheep fed on corn, and also reduced silver nitrate. Beef tallow from cattle fed on the meal also showed a high melting-point and responded to Becchi's test.

Many experiments have shown that a ration of cottonseed meal tends to make a hard butter, to increase its index of refraction and iodine value, and to raise its melting-point. There seems but one way of reconciling these apparently contradictory results—appearing contradictory because unchanged cottonseed oil, or oleïn or oleïc acid produced by its metabolism would raise the iodine value, but would also lower the melting-point instead of raise it. We must, therefore, conclude that the oil in passing from the circulation into the milk must undergo a change in the mammary glands whereby not only is oleïn or oleic acid pro-

[1] Texas Expt. Sta. Bull. No. 29, pp. 349-355.

duced, but also some fat having a much higher melting-point. We believe this theory to be generally accepted as an explanation of the effects produced upon butter by cottonseed oil. It would seem, from the facts brought out by the work of Harrington and Adriance, that some similar theory is required to explain the effects of cottonseed oil upon body fat, although their results were not uniform. Their conclusion was that, in general, the feeding of cottonseed meal seemed rather to lower the iodine value of the animal fat, although its melting-point was raised, and it responded to the test for cottonseed oil. This conclusion (which is fairly well substantiated by other workers) naturally suggests that the oil in cottonseed meal does not pass from the blood through the walls of the capillaries into the cells of the connective tissue unchanged; neither are those products of its metabolism which finally enter the fat (if any of them do enter it) richer in olein or oleïc acid than the animal fat itself, else its iodine value would, of necessity, be raised. It would also follow that while the oil itself does not enter the fat unchanged, yet the color-producing substances contained in it do not undergo any transformation before their deposition in the fat, inasmuch as their activity still remains.

Langfurth[1] investigated American lard, rendered from the fat of hogs which had been fed on cottonseed meal, and found that Becchi's reagent was completely reduced; that nitric acid (sp. gr. 1.4) gave a mahogany-brown color, and that Halphen's reagent gave a dark onion-red coloration, equivalent to that given by 30 per cent. of cottonseed oil. (In view of our experience, we should like to know how the comparison showing this large percentage equivalent of color was made.) It is thus seen that the nitric acid test, as well as Beccchi's and Halphen's reagents, indicate the presence of a pretty large amount of oil in the lard, and yet Langfurth states that its analytical numbers were normal—a statement somewhat difficult to reconcile with the color tests. It certainly would be impossible for a lard to contain 30 per cent. of cottonseed oil and still have analytical numbers that are normal for pure lard. This would be especially true of the iodine value and the melting-point.

The observations of Langfurth are also interesting from another point of view. Raikow[2] has shown that the active prin-

[1] *Ztschr. angew. Chem.*, **14**, 685-686 (1901).

[2] *Chem. Ztg.*, **24**, 562-563 (1900).

ciple in cottonseed oil to which the reduction in Becchi's test is due is not the same as that which causes the coloration with Halphen's reagent, and a number of workers have shown that heat destroys the ability of the oil to react with either of these reagents. It is also stated[1] that the substance to which the coloration with nitric acid is due is not affected by a temperature of 240° C. It therefore follows that these three color reactions are due to the presence of three distinct substances in the oil. According to Langfurth's observations, therefore, all of these different principles to which color reactions are due are accumulated in the animal fat, but none of its analytical constants are modified thereby.

The logical deduction to be drawn from these facts, brought out by Soltsien and Langfurth, is that in case of hogs, at least, cottonseed oil is not deposited in the body fat unchanged, but that some kind of metabolism occurs by which certain color-producing bodies are absorbed and deposited unchanged, while phytosterin and the other constituents of the oil, to which its chemical and physical constants are due, are not thus absorbed and deposited.

A consideration of all the statements and opinions, outlined in the foregoing pages, leads to much confusion of mind as to the real facts in the case. Doubtless if our knowledge of the substances to which the color reactions are due were more extensive, some of these anomalous, and, to our mind, contradictory results would be less confusing. It hardly seems to us probable that metabolic processes will result in causing the three distinct chromophoric bodies in cottonseed oil to be deposited unchanged in the fat, while such an important constituent as oleïc acid and such a characteristic one as phytosterin either find their way elsewhere or are not deposited in the fat until after they have undergone such profound changes that their metabolic products exercise no influence upon its analytical constants. Certainly much more experimental evidence concerning the point is extremely desirable. It is our intention to make a thorough study of the lard samples obtained in the experimental work described in this paper, with the hope of throwing some light upon this puzzling chemico-physiological problem.

[1] Benedict and Lewkowitsch's "Oils, Fats, and Waxes," p. 310 (1895).

SUMMARY.

(1) An investigation was undertaken with the view of determining to what extent the lard from cottonseed meal-fed hogs would give a coloration with Halphen's reagent.

(2) Twenty-three animals were used in the experiment, being divided into four lots, as follows: Six in lot I; six in lot II; eight in lot III; three in lot IV. Lot I received one-fourth pound cottonseed meal per animal per day; lot II one-half pound per animal per day; lot III an amount approximately equal to one-fifth of the grain ration; while lot IV received all the animals could be made to eat.

(3) One animal in each lot, except lot IV, was killed at the end of the second, fourth, sixth, eighth, tenth and twelfth weeks. Two of lot IV were killed at the end of fourteen weeks. Of the two animals remaining in lot III one was killed three months and the other five months after the close of the feeding period.

(4) The feeding period began January 29, 1903, and closed April 23, 1903, thus covering a period of 84 days.

(5) The minimum amount of cottonseed meal eaten by any individual was 7.7 pounds and the maximum, 154 pounds. The minimum amount consumed by any individual per one hundred pounds body weight was 1.8 pounds, and the maximum, 75 pounds.

(6) Lard was rendered from samples of fat taken from each animal, from kidney, jowl, back and intestines, and in many cases also from the belly.

(7) All lard samples gave a distinct and, in some cases, a very strong coloration when treated with Halphen's reagent. The coloration equivalent, expressed in percentages of cottonseed oil, ranged from 0.4 to 15 per cent.

(8) In general, the greatest degree of coloration was found in kidney fat lard, and the least in intestinal fat lard. In a majority of samples back fat lard gave a greater intensity of color than that from jowl fat. The color-producing principle of cottonseed oil is transmitted to all parts of the animal, although in unequal amounts.

(9) If the degree of coloration with Halphen's reagent is to be expressed in terms of percentage of cottonseed oil, the kind of oil used for comparison, and the conditions under which the

comparison is made should be stated, because different oils respond with unequal intensity, and the depth of color increases after cooling.

(10) When the color-producing substance is once deposited in the fat of hogs, it is exceedingly persistent, as illustrated in the case of animals Nos. 7 and 8 in lot III, No. 7 being killed July 26th, three months after it had received its last ration of cottonseed meal, and lard rendered from its fat showing a coloration equivalent to 4 per cent. of cottonseed oil and No. 8 being killed September 20th, having eaten no meal for five months, and a composite sample of its fat yielding lard which showed a coloration equivalent to 3 per cent. of oil.

(11) Other investigators have shown that the three different color-producing substances in cottonseed oil, to which are due the reactions with Halphen's and Becchi's reagents, and also with nitric acid, are deposited unchanged both in the body fat of different animals and in the secreted fat of cows. A number of experiments with the feeding of cottonseed meal have shown its effect to be a marked modification of the properties of the body fat, while others indicate that a large amount of these color-producing substances may find their way unchanged into the body fat, while its chemical and physical constants remain the same, one investigator finding in lard a coloration with Halphen's reagent equivalent to 30 per cent. cottonseed oil, and yet its analytical numbers were normal. While the evidence is somewhat conflicting, we are rather unwillingly led to the conclusion that probably the color-producing principles are separated from the cottonseed oil by metabolic processes, and that the effects of metabolism are so profound that its final products, if they find their way into the fat, do not modify it in any important degree.

ACTION OF SOAP ON CALCIUM AND MAGNESIUM SOLUTIONS.

By V. H. Gottschalk and H. A. Roesler.

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While working with Clark's well-known method for the determination of hardness of water, certain phenomena were observed, which it is the object of this paper to describe.