Of course, it will be understood that these criticisms in no sense lessen the value of the contributions of de Chalmot, and his fellow specialists in the subject of the pentosans, to the general problems of assimilation and metabolism. Any error which may have been made is one only of interpretation. The too free use of "pentose" and "pentosan" as the equivalent of "furfurol-yielding constituents" will prove, we think, to have temporarily obscured some important points in the physiology of the elaboration of plant tissues, but there being now a substantial agreement in the main issue it may well be left to time and experimental investigations to "materialize" this further chapter in the life of the plant cell.

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THE SEPARATION OF SOLID AND LIQUID FATTY ACIDS.

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THE fatty acids insoluble in water, which constitute the greater part of most natural fats, are probably always found as a mixture of two groups, one composed of saturated, the other of unsaturated compounds, the former belonging to the acetic series and the latter to the acrylic and other series containing still less hydrogen. In most fats of commercial importance, such as tallow, lard, cottonseed-oil, and some other vegetable oils, the first named group is represented by only two members, stearic and palmitic acids, which are solid at ordinary temperatures, while the last contains the liquid oleic and linolic acids.

An accurate separation of these groups ought to be the first step in the analysis of any fat, as without it other quantitative reactions, such as the amount of iodine absorbed or alkali combined by the fatty acids, would lead to no very definite conclusions regarding the actual composition of the mixture.

Although a number of analytical methods have been proposed to obtain this separation, their accuracy has not been without question, while in my work I have, at different times, attempted to separate solid from liquid fatty acids and entirely failed to obtain satisfactory results. It therefore seemed to me very desirable to make a study of some of the methods which have been published, together with any modifications which might suggest themselves in the course of the work. In the following experiments it was attempted not only to compare the different methods, but to determine, if possible, by some direct test, the degree of accuracy of each.

Varrentrapp observed that the lead soap of the solid and liquid fatty acids show great difference in respect to their solubility in ether, soaps of the liquid acids being readily soluble, while those of the solid acids are almost, if not quite, insoluble. On this property of lead soaps have been based the methods which seem to promise the best results, though other solvents than ether have been recommended.

1. Perhaps the most carefully worked-out process is that of Muter, described in the *Analyst* of April, *1889*, which has been employed in examining lard chiefly for the purpose of detecting an adulteration of cottonseed-oil or stearine. This method has been examined and slightly modified by Nathaniel J. Lane, who has published his results in this JOURNAL, February, *1893*. According to this process, the neutral potassium soaps of the fatty acids in boiling water are precipitated by lead acetate. The lead soaps which adhere to the sides of the vessel are washed several times with boiling water, dried as quickly as possible, and then boiled with redistilled ether and allowed to cool thoroughly, filtered, washed, and the filtrate, containing the lead soaps of liquid acids, decomposed with hydrochloric acid and water. The ethereal solution of fatty acids floats on the acid liquid. An aliquot part of this solution is titrated with standard sodium hydroxide to determine the percentage of liquid acids, and another part is evaporated and dried in a current of carbon dioxide and treated with Hübl's solution. Muter finds about ninety-four per cent. as the amount of iodine absorbed by the liquid acids of lard.

Having carefully prepared the insoluble fatty acids of lard rendered in my laboratory (saponifying in the usual manner and decomposing with hydrochloric acid while avoiding exposure to air as much as possible), I carried out the process of Muter, following carefully all important details. A part of the ethereal solution of liquid fatty acids obtained was put into a weighed Erlenmeyer flask provided with a ground-glass stopper; then dried on the water-bath in a strong stream of carbon dioxide, which had been washed by passing through sodium bicarbonate solution and dried over calcium chloride. After thoroughly cooling the flask, dry air was drawn through for a few minutes to remove carbon dioxide, and the flask weighed. The percentage of iodine absorbed by these fatty acids was then determined in the usual manner, the operation being conducted in the same flask. The object in finding the iodine absorption was to determine, at least comparatively, the purity of the liquid fatty acids, *i. e.*, their freedom from saturated compounds. The following iodine numbers were found:

	Iodine absorbed. Per cent.
Lard	56.27
Fatty acids of lard	58.49 59.26
Liquid acid by above process	94.06

In order to test the solubility of lead stearate and palmitate in ether, a mixture of stearic and palmitic acids, such as is found in commercial stearic acid, was purified by crystallization from alcohol till it gave an iodine number equal to zero. The lead soap was then prepared by the above-described method and a small quantity boiled twice with alcohol to remove free fatty acids and then treated with boiling ether and allowed to cool. At 25° C. 100 cc. of ether dissolve 0.015 gram of the lead soap, and this does not crystallize out at 0° C. This would cause an error of about one per cent. in the above process, while it is possible that, in the presence of lead oleate the solubility of the stearate and palmitate might be much greater.

Another and more serious objection to Muter's method consists in the unavoidable exposure of the lead soaps to the air. All my results seem to show that the lead soaps of the unsaturated fatty acids oxidize even more readily than the free acids. So that, though great care is taken to remove the liquid acids from the air, it does not seem possible to treat lead soaps in this way without a good deal of oxidation, especially during the tedious filtration. Fahrion has noticed this remarkably rapid oxidation in the case of calcium soaps (*Chem. Ztg.*, *1893*, 17).

2. In his book, Chimie Analytique des Matières Grasses, Jean describes a method of examining lard. This method—modified in the manner of treating the liquid acids—was next employed. Four grams of the same lard fatty acids were dissolved in fifty cc. of ninety-five per cent. alcohol; and to this was added two and a half grams of lead acetate dissolved in twenty cc. of the same alcohol, both solutions being hot. There was an immediate precipitate, and the mixture was allowed to stand one hour at the temperature of the laboratory, and then cooled to 15° C. and kept at this temperature one hour longer. A part was then filtered into a separatory-funnel, treated with ether and dilute hydrochloric acid, washed, and dried in carbon dioxide. The iodine number of these liquid fatty acids was found as in the previous experiment, and their percentage in the original solution calculated. The precipitate was washed thoroughly with ninety-five per cent. alcohol, decomposed with hydrochloric acid, and the solid fatty acids separated and weighed. Their iodine number was also obtained. The following were the results:

	Per cent. obtained.	Iodine absorbed. Per cent.
Solid fatty acids	46.24	4.90
Liquid fatty acids	51.82	103.37

These figures indicate a fractional precipitation in which all the solid and a part of the liquid acids are precipitated. The iodine number of the solid acids and the low yield of liquid acids both point to this. That part of the liquid acids in the solution is probably held there by the acetic acid liberated. This must be the case as lead oleate is only slightly soluble even in absolute alcohol.

If the liquid acids,precipitated along with the solid, consisted entirely of oleic acid as was probable, the iodine number of the liquid part of the fatty acids would be somewhat lower than 103.37 per cent. Yet the amount of liquid acids precipitated being in this case quite small, could only reduce the result by two per cent. at the most. The great difference between this iodine number and that of the liquid acids obtained by Muter's method show that the latter must have contained a considerable amount either of saturated or of oxidized fatty acids.

Jean gives the iodine number of the liquid acids of lard as 92.71. This low figure may be accounted for by the fact that he does not take the precaution to dry the fatty acids in a current of carbon dioxide.

3. It appeared desirable to determine first, whether the liquid acids obtained by the last process were entirely free from solid acids, and second, whether the precipitation was actually a fractional one, more or less of the liquid acids precipitating according to circumstances of temperature, concentration, etc. In order to decide these questions, four grams of lard fatty acids (prepared at another time and from another sample of lard) were dissolved in ninety-five per cent. alcohol and precipitated with lead acetate, cooled for one hour to 15° C., and filtered. Ten cc. of the filtrate were drawn off, decomposed with hydrochloric acid, the fatty acids dried in carbon dioxide, and treated with Hübl's solution as before, while the remainder of the filtrate was cooled for one hour to 0° C. At this temperature there was an additional precipitate. This precipitate was filtered off and washed with ninety-five per cent. alcohol, the washings being thrown away. The fatty acids obtained from the filtrate were also treated with Hübl's solution. The iodine absorbed by the fatty acids of these fractions was as follows:

	Amount obtained.	Iodine number.
Lard fatty acids		62.57
Fatty acids from filtrate at	15° 0.2675 gram (46.81 %)	109.35 per cent.
" " " precipitate at	0° 0.1020 "	
" " " filtrate at	0° 0.1915 "	118.02 per cent.

The fatty acids obtained from the precipitate at 0° chilled at 0° C. and melted at 7° C. It could therefore contain little or no solid acid, and was probably pure oleic acid. Its iodine number was not determined, but calculating from the iodine number of the filtrate at 0°, it must be very nearly 90—that of oleic acid—to make the iodine number of the mixture 109.35.

It is plain from the above results that the precipitation of fatty acids by lead acetate in alcohol is not a quantitative separation but a fractional precipitation, in which the solid acids are precipitated first, then oleic acid, and finally linolic acid.

Though no quantitative separation of the liquid acids has been made, their percentage can be calculated as follows: The percentage of liquid acids in the alcoholic filtrate is calculated from the fraction drawn off. This is multiplied by the iodine number of these fatty acids. The result is deducted from the iodine number of the original fatty acids. The difference represents oleic acid. Dividing this by 0.9 gives the percentage of oleic acid precipitated with the solid acids. Adding this to the liquid acid in the filtrate gives the total liquid acid. In the case of the last sample the calculation is: 46.81 per cent. × 109.35 per cent. = 51.19. Deducting from 62.57 gives 11.38. Divide by 0.9 = 12.64, oleic acid in precipitate; add to 46.81 gives 59.45, total liquid acids; whose iodine number is $\frac{62.57}{59.45} = 105.2$.

The linolic acid is easily calculated from the iodine absorption of the liquid acids—109.35 represents 78.5 per cent. oleic acid, 21.5 linolic acid. 21.5 × 46.81 = 10.06 per cent. linolic acid in the original fatty acids.

4. A process described by Röse (*Repert. f. analyt. Chem., 1886*) consists in shaking the ethereal solution of fatty acids with finely powdered litharge, when the lead soaps are formed and those of the liquid acids remain in solution. This method was next employed, using not ether, however, but petroleum ether, completely volatile at 80° C., in which I had found lead stearate and palmitate to be much less soluble than in ether. 100 cc. of petroleum ether at 30° C. was found to dissolve 0.0034 gram of the mixed soaps while at 5° only 0.0018 gram were dissolved. Two and a half grams of the lard fatty acids used in the last experiment were dissolved in fifty cc. of petroleum ether, two and a half grams of lead monoxide added, and allowed to stand two hours in a warm place with frequent agitation. A fifty cc. flask was used having a graduated neck. It was filled almost to the stopper in order to avoid the oxidizing effect of the enclosed air. After two hours time the yellow lead monoxide had become white in appearance, due to the formation of lead stearate and palmitate, and the precipitation was judged to be complete. It is clearly not necessary to combine the lead with all the fatty acid. If a part of the liquid acids are in the form of lead soaps this will be a guarantee that all the solid acids have combined. The presence of lead in the filtrate will show

whether the agitation has been continued long enough. Before filtering, the flask was placed in ice-water for half an hour in order to insure as complete a precipitation as possible. Then it was filtered, a definite part of the original volume pipetted off, decomposed with hydrochloric acid in a separatory-funnel, and evaporated and dried in a current of carbon dioxide as before. The liquid acids calculated to the whole solution yielded 55.10 per cent. having an iodine number of 108.66. The precipitate on washing then decomposing with hydrochloric acid gave 44.70 per cent. solid acids having an iodine number of 3.02 per cent. This impurity was probably caused by an oxidation of the liquid acids to compounds whose lead soaps were insoluble in petroleum ether.

The results obtained here are not far from those calculated by the alcohol method. The petroleum ether method would have the advantage over the other in that the iodine number would be that of the liquid acids and not only of a fraction from which part of the oleic acid has been precipitated. On the other hand a quantitative result by the petroleum ether method could only be arrived at where the fat is perfectly fresh and the greatest precautions have been taken to avoid oxidation during the whole manipulation. The same sample of lard fatty acids, after standing seven days in a closed jar, gave the following results :

	Iodine number.
Solid acids	10.1 per cent.
Liquid "	101.7 "

The oxidation had given products both soluble and insoluble in petroleum ether ; and in this case the percentage of liquid acids cannot be inferred from the analysis.

A precipitation of all the solid and part of the liquid acids by lead acetate in alcohol, by the method described by Jean, with the addition I have proposed of finding the iodine number of both the original fatty acids and those in the solution, and calculating therefrom, would give accurately the percentage of solid and liquid acids in a fat. Such a method would perhaps be too cumbersome for general commercial work. Still I believe it is at present the only positive solution of the problem.