# New Aspects of the Chemistry of the Oils and Fats

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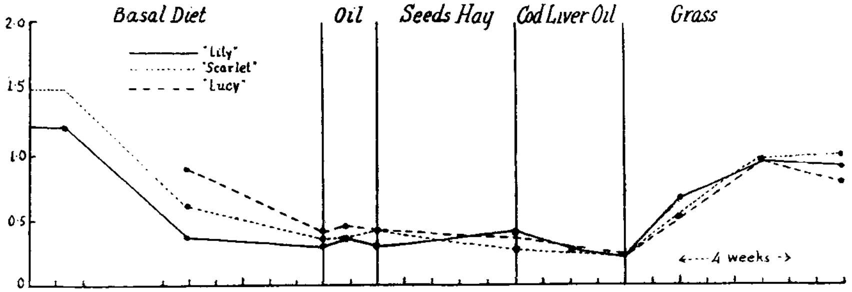
THE title of this paper is misleading, because I propose to interpret the word "fat" in its wider sense, as meaning the natural occurring product from plant and animal sources and then focus attention on those substances which have been sadly neglected by the chemist which were noted by Chevreul to resist the action of alkalies.

Most of the common fats contain 98 to 99% of glycerides and, in addition, certain other substances. Of these, some, such as esters of the high alcohols, cholesterol, etc., are saponifiable while others are not affected by alkalies. Complete saponification of the fats and subsequent extraction of the soap solutions by a suitable solvent removes a fraction, which we term "unsaponifiable" matter, which has been little studied by the chemist in the past beyond the quantity present, while the physiologist has formerly concentrated his attention on the digestion, absorption, and biological degradation of the glycerides.

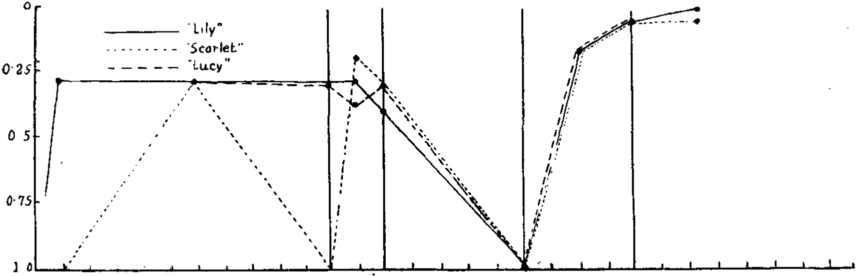
The bio-chemist will find in the unsaponifiable matter a true El Dorado for research.

Thudichum was the first to examine various pigments such as occur in butter, egg fat and palm oil, and in 1869, published a paper wherein he termed them "Luteins."



*Ordinates give arbitrary but comparative units of Color*

Vitamin Activity



*Ordinates give the daily dosage of butter necessary to restore normal growth to rats on a diet deficient in vitamin A*

He showed one of these pigments to be identical with that found in the root of the carrot, now known as "Carotene."

Willstatter and his colleagues isolated a number of these pigments which are as follows:

| Pigment | Formula | Chief Sources |
|---|---|---|
| Carotene | $C_{40}H_{56}$ | Green leaves, carrots |
| Xanthophyll* | $C_{40}H_{56}O_2$ | Green leaves |
| Lycopin | $C_{40}H_{56}$ | Tomato |
| Fucoxanthin | $C_{40}H_{56}O_6$ | Brown seaweeds |

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* It is believed that a number of isomeric xanthophylls exist. They have not yet been separated from each other in crystalline form, but on the evidence of the spectroscope their existence is suspected.

The first two are found widely distributed in the plant world. In all cases, they are readily oxidized. For this reason, great care must be taken to separate them. They are found in several animal products, such as butter, fat, most body fats, and the yolk of many types of egg.

It is generally known that milk from the herd of cattle from pasture in summer if the crop is growing well will have a richer color than when the animals are brought into the stalls in winter, especially when deprived of green foods.

Professor L. S. Palmer of the University of Minnesota, found that the natural coloring matter of butter and egg yolks are derived from fat soluble pigments of the food.

During the digestion of foods containing the lipochromes, as these pigments are called, tend naturally to be absorbed in association with the fats and tend to follow them during transport and storage in the animal body.

The notable exception is in the case of the pig, whose fat never shows any appreciable yellow color.

It is only natural that in the process of storing fat for forming milk fats or egg yolks that some of the pigments of the food will be deposited along with them.

In these experiments the cows were brought into stall from the meadows and were fed on a ration, typical of many winter farm diets, consisting of wheat and maize gluten as concentrates, mangolds, and well-cured hay. Such a ration is very poor in lipochrome pigments and, as you will observe, the color of the butter soon diminished, only rising again in intensity when the animals were once again turned out to grass.

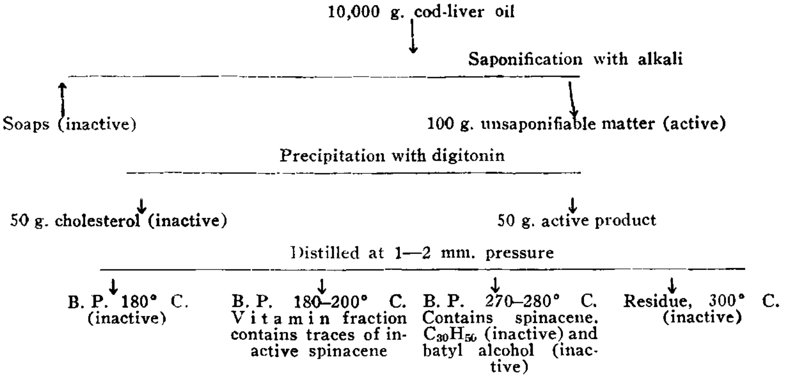
The association of the yellow coloring matters with butter fat may, in a sense, therefore be regarded as accidental, and it may be imitated by the experiments which have led to the production of these rather startling eggs (which were exhibited). The birds that laid these eggs were maintained on a ration deficient in the natural fat-soluble pigments until the marked yellow color of the yolks at the commencement of the experiment had completely disappeared. As soon as they were laying eggs with almost colorless yolks some fatty material deeply pigmented with the fat-soluble dyes Sudan III and Nile Blue were mixed with their food. The absorption of the dyes and their deposition in the yolk followed, I think, from purely physical causes, such as their partition between oil and water phases. These experiments leave unanswered the main question I wished to raise, namely, what is the significance of the lipochromes in the organism? It is true that the white-yolked eggs I have shown you may be fertile, and on incubation produce healthy chicks, incidentally with white and not yellow downy feathers, but in spite of this, and the considerable amount of evidence that the presence of the pigments is accidental, one feels some reluctance in abandoning the view that Nature, in most respects a strict economist, has colored an egg yolk yellow for a definite purpose. In the green plant there seems no question but that the pigments play an important part in the chemical changes that occur in the chloroplast. The very relationship that exists between the formula of the hydrocarbon carotene, $C_{40}H_{56}$, and that of xanthophyll, $C_{40}H_{56}O_2$, suggests at once to the mind of a chemist an oxidation-reduction system capable of functioning in an oily phase. In spite of much speculation, however, little is yet known of their function in the plant, and nothing whatever of their possible significance in the animal.

A short time ago there was no little excitement in biochemical circles because Prof. Steenbock, in America, traced a most remarkable parallel between the occurrence of the lipochromes and that of the vitamin A; green leaves, carrots, yellow maize, yellow butter, many other food products, all seemed to show a vitamin potency that could be correlated with their degree of pigmentation. The attractive idea that one of the pigments might actually be the vitamin seemed too good to be true, and it was, unfortunately, disproved by tests of the pure substances on animals. It is doubtful, therefore, whether the association is of more significance than physical causes; *e.g.*, similar solubilities, would account for. Thus, for example, the administration of cod-liver oil, itself poor in lipochromes, but rich in vitamin A, raised the food value of the butter fat of the experimental cows without appreciably affecting the color.

Let me now turn to the fat-soluble vitamins themselves, because they are amongst the most important of the constituents of the unsaponifiable matter of the fats in which they are naturally present. The beneficial influence of cod-liver oil in the treatment of wasting diseases, general malnutrition, and rickets, is, of course, familiar. At one time its action was believed to be due to the small traces of iodine that are frequently found in the oil, particularly in the cruder, dark varieties. Another theory, also widely held at one time, attributed the nutritious qualities to the peculiar nature of the unsaturated fatty acids which it contains, and which, it was held, would be more readily assimilated by the enfeebled organism than those which occur in most other fats.

Both theories have been to a large extent rendered untenable by the discovery that the influence of growth and bone formation of the oil itself may be exerted in exactly equivalent amount by the material that can be extracted from the soaps after saponification, provided care is taken during the process to minimize the chances of oxidation occurring. The physiologically active fraction is a yellow crystalline waxy product, representing just under 1 per cent of the original oil, and is devoid of iodine. The 1 per cent unsaponifiable fraction from cod-liver oil has formed the basis of a number of attempts to determine the chemical nature of the active substances. The discovery of the stability of the vitamins to the somewhat drastic process of saponification was for many

FIG. 2



of us a great relief, because at the back of our mind during our earlier researches we had always held the fear that the units responsible for the effects on the animal organism might be ill-defined, unstable substances, such as the toxins and enzymes are believed to be, and would, therefore, prove extremely difficult to investigate by chemical means with the view to discovering their character.

Figure 2 outlines the main paths by which we have recently attempted to gain light as to the nature of the one principle which seems so necessary for the satisfactory growth of the young animal. The very highly active fraction obtained by fractional distillation at reduced pressure appears to consist to a large extent of higher alcohols with unsaturated molecules, probably open chain in nature. Whether such an alcohol is the active principle or not cannot be said until purer preparations than those that have yet been made are available —the claim recently advanced by the Japanese investigator Takahashi and his colleagues, to the effect that they have isolated the active substance in the pure state and shown it to be an unsaturated alcohol, "biosterin," $C_{27}H_{46}O_2$, cannot be admitted, as a critical examination of their work proves that their preparations were of doubtful purity. The investigation of the unsaponifiable matter from fish liver oils has revealed the fact that higher alcohols are frequently found there, and we have recently been able to show that of those of which the identity has been reasonably well established, namely, selachyl alcohol, $C_{20}H_{40}O_3$, oleyl alcohol, $C_{18}H_{36}O$, batyl alcohol, $C_{20}H_{42}O_3$, and the well-known alcohol, cholesterol, $C_{27}H_{46}O$, no one can be identified with the active principle.

The idea that vitamin A might well be an unsaturated alcohol of this type is not unattractive in some respects, if only for the reason that it seems to yield a number of color reactions, some of which are now being investigated for their value as means of detecting and estimating vitamin A in foodstuffs, which in their general character resemble those which are given by the sterols, the universally distributed higher alcohols believed to be related to the terpenes.

The presence of cholesterol, phytosterol, or similar substances in the unsaponifiable matter or plant or animal fats calls attention to the remarkable extent of our ignorance concerning the function of these substances in the living cell. The literature is congested with a mass of wholly unreliable work on these substances, and no problem in biochemistry calls more urgently for solution than that which concerns their significance in the cell. Even their structures, investigated with great care and precision by Windaus and others for a number of years, have so far resisted all efforts at elucidation. To one *rôle* of cholesterol and the closely related compound phytosterol I would like to draw attention, for it concerns our discussion of the fat-soluble vitamins; incidentally, the story of its discovery is, I think, one of the most interesting that recent scientific advance can provide.

Almost every conceivable type of causative agent has at one time or another been blamed for the onset of rickets, but, broadly speaking, the theories of its etiology can be divided into two main groups.

Of these, one consisted of a variety of views all of which held that in some manner or another defective diet was responsible for the trouble, whilst the other type of theory was based on the belief that faulty hygienic surroundings, in particular, lack of fresh air and sunlight, were the main causative agents.

The supporters of the two contending schools of thought each, in turn, brought forward evidence which to the outside observer seemed equally convincing and for a time no reconciliation seemed possible. In 1916 Huldschinsky demonstrated that rickets in infants could be cured by exposure to the light of a quartz mercury-vapor lamp, well known to be a source of light rich in the short wave-length rays of the ultra-violet part of the spectrum.

Confirmaton of this fact was soon forthcoming, and especially by the researches of Dr. Harriette Chick and her colleagues in Vienna, just after the War, was it shown that the cure of rickets may be achieved with equal success either by such exposure or by the administration of certain foods, such as cod-liver oil.

These facts at first seeming to stand in somewhat uncertain relation to each other, were, in a short time, correlated by the interesting observations of the American investigator Steenbock, who found that the production of rickets in animals by feeding them on a diet deficient in certain constituents could be prevented by previously exposing the food to the rays of an ultra-violet lamp.

At once many of the apparent inconsistencies between the results of the earlier work seemed to disappear.

One by one the constituents of the diet were examined, with the result that the proteins, carbohydrates, and salts were found to be unaffected by the action of the rays in so far as the formation of any substance possessing anti-rachitic action was concerned. When, on the other hand, the fats were examined, a very different story was revealed, for its was found that a number of inactive oils, such as linseed or cotton-seed oil, which in the untreated form exhibit no anti-rachitic powers, became strongly curative after exposure to the light of the quartz mercury-vapor lamp for an hour or two.

Almost simultaneously in this

country and in America the reason for this was discovered, by the observation being made that the precursor of the active substance occurred in the unsaponifiable fraction of the original oil, and that it was actually the sterol.

Specimens of cholesterol or phytosterol, substances which, as you are aware, can be prepared in a state of considerable purity, and which themselves possess no apparent action on the process of deposition of calcium salts in growing bone, assume that property to a marked degree after exposure to the rays of an ultra-violet lamp for comparatively a short time. As you can see from the two specimens before you, the "activated" cholesterol, as we may term it, has lost the beautiful clean white color and well-marked crystalline form of the untreated sample. It shows a depressed melting-point, 143.5° instead of 149.5°, and gives rise to color reactions differing quite appreciably from those of the unchanged material. These facts lead us to believe that the change is a photochemical one. The activation proceeds both in the absence and presence of oxygen, and after as short a time as two hours a sample of cholesterol, provided it is suitably exposed, becomes strongly anti-rachitic. The activated material has as yet been very little examined chemically. The anti-rachitic principle has been separated from the much larger bulk of unchanged cholesterol by the precipitation of the latter substance with digitonin, but the amount obtained was insufficient to be of any value for chemical examination. You will now be in a position to understand why we believe that a reconciliation between the two theories, or groups of theories, regarding the etiology of rickets has been achieved. The similar action on the forming bone of exposing the child's skin to sunlight or the rays of another source of ultra-violet light, or, on the other hand, of administering cod-liver oil, is probably due, we think, to both treatments supplying the same necessary factor vitamin D. In the one case the vitamin is synthesized in the skin from cholesterol, or a closely related substance, by the photochemical action of the rays in the vicinity of $\lambda = 300\ \mu\mu$, whilst in the other the missing factor is supplied direct in the oil.

Let me now turn to the third of the fat-soluble vitamins, spoken of as vitamin E, and discovered quite recently by Prof. H. E. Evans, of Berkeley University, California. This observer noticed that, although young rats would grow to maturity at a normal rate when fed upon an artificial diet containing a balanced mixture of proteins, fats, carbohydrates, salts, and the vitamins A, B, C, and D, they were incapable of showing a normal reproductive power until the ration had been supplemented with certain other foodstuffs.

The distribution of the anti-sterility factor, as it has been termed, in natural foods showed that it could not be identified with any one of the then recognized dietary essentials. Accordingly it was regarded as a new member of the vitamin class and given the distinctive label E.

It is present to a relatively marked extent in the oil that can be expressed or extracted from the germ or embryo of wheat, and, as Evans and Burr have just shown, it survives saponification of that oil and passes into the unsaponifiable fraction. It appears to be the most stable of the vitamins, for it is unaffected, at least as regards its action on the female rat, by heat, oxygen, or reduction with hydrogen. From the unsaponifiable fraction of wheat oil the cholesterol has been removed without lowering the activity of the material, and the residue has been fractionally distilled; the physiologically important substance passes over at a temperature of about 180° (2 mm.). Nothing is yet known as to its chemical nature.

It may interest you to be given a few of the doses of active preparations of the different fat-soluble vitamins that have been found to satisfy the requirements of the animals used in the tests:—

| Vitamin | Rats, Body Weight in g. | Minimum Dose Recorded in g. | Dose Per 100 g. Weight |
|---|---|---|---|
| A ....... | 100 | 0.00001 | 0.00001 |
| D ....... | 100 | 0.0001 | 0.0001 |
| E ....... | 200 | 0.0005 | 0.00025 |

Of course, it must be admitted that the comparison is to a large extent unjustified, if only because the preparations of the vitamins used were in all cases far from being the active substance in pure state; nevertheless, it is curious that these approximations are of the same order of magnitude.

My reference to Evans' discovery of a vitamin playing an important part in maintaining the delicate balance in the organism on which the reproduction of the species depends calls to my mind another substance with what seem to be not dissimilar properties which also plays a part in that respect. The phenomenon of œstrus or "heat" in the female animal presents many curious and fascinating problems to the physiologist and biochemist, but few of them are, to my mind, of more outstanding interest than that which concerns the mechanism by which the ovaries periodically control the appearance and duration of the characteristic changes in the uterus.

This control is known to be exerted by means of an internal secretion liberated from the ovarian tissue into the circulating blood, for œstrus is at once suppressed by removal of the ovaries.

The chemical substance responsible for these effects can be extracted from the ovaries by certain solvents, and will exert the characteristic action on injection into an ovariotomized animal.

The active principle is a fatty substance, and appears—although this point requires confirmation—to withstand saponification and to pass into the unsaponifiable fraction. The statement has been made that it can be distilled at greatly reduced pressures without serious loss. Once again the biochemist must deplore his ignorance, since the chemical nature of this most interesting substance is quite unknown. Do not, as a body of chemists, treat him with scorn; do not yield to the tendency, marked in some quarters, to pour ridicule on the slimy messes which it is the misfortune—in my heart of hearts I believe it to be a good fortune—of the biochemist to have to deal with.

Remember that over 100 kg. of cod-liver oil had to be saponified, and the unsaponifiable matter extracted in an inert atmosphere, before as little as 300 g. of a preparation suitable for fractional distillation could be obtained; bear in mind that the ovaries of many thousand sheep or pigs must be collected and extracted before a miserable 100 g. of fatty material containing the ovarian hormone can be gathered together; appreciate that at every stage of the fractionation animal tests requiring three to six weeks may be necessary before the fate of the active substance during the last process that was applied to it can be known. I counsel you to respect these facts lest a worse fate befall you.

In conclusion, let me tell you another curious story in which I and my colleagues are now taking an active interest.

All of you know that the liver acts, in one of its many capacities, as a storage organ of either fats or reserve carbohydrates in the form of glycogen. That is all very well, but I confess my sense of things as they should be in the biological world was greatly surprised when it was shown that certain fish habitually pass their days carrying in their livers as much as 50% of the weight of that organ in the form of a liquid hydrocarbon. This remarkable fact, originally discovered by Mastbaum, but later more fully studied by Tsujimoto and by Chapman, led to the discovery of the hydrocarbon now termed squalene (spinacene) $C_{30}H_{50}$. Its molecule possesses six ethylene bonds, and appears to be an open carbon chain related to the terpenes. Recently in my laboratory Mr. H. J. Channon has discovered another hydrocarbon, apparently of a type resembling that to which squalene belongs, in mammalian livers, but in amounts very much smaller than the quantities of squalene that may be found in fish livers. The significance of these hydrocarbons is as yet unknown, but is being made the subject of study in our laboratories. The amounts present in fish livers may show great variation. Some of the elasmobranch species contain as much as 80% of the total fat of the liver in the form of squalene, whilst other species, often closely related biologically, may only possess a trace.

I have attempted in this paper to present to you an aspect of the chemistry of the fats which possibly may not before have been brought to your notice. The study of the many and curious substances that are found in the unsaponifiable matter of various oils and fats that occur in plant and animal tissues is crying out for attention. For this reason I hope I have interested some of you in what is, to me, a field of chemical research offering problems of fascinating nature and far-reaching importance.

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## Changes in Olive Oil After Long Standing

(*Continued from page* 140)

mal affinities for iodine of the "unsaturated" oleic and other fatty acids present. The acidity (and Acid No.) has increased about sixteen-fold and undoubtedly much more liberated free fatty acids have been changed to non-acid substances, possibly ketones and aldehydes. Just why the Ester No. (and incidentally the Saponification No.) have increased is not so plain, in view of the fact that there must have been a great loss of glycerol esters during the liberation of free fatty acids.

The data suggests that a hasty condemnation of an old olive oil as "adulterated" is possible and that the history of an abnormally testing oil should be carefully ascertained before prosecution is started.