ON THE SULFURIC ACID REACTION OF BUTTER FAT AND
THE DISAPPEARANCE OF THE REACTION FROM
VITAMIN A-CONTAINING BUTTER FAT
THROUGH THE ACTION OF OXI-
DIZED FAT.

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(Received for publication, October 16, 1924.)

In 1922 Drummond and Watson published investigations on the sulfuric
acid reaction of liver oil. They found that the reaction was not only yielded
by cod liver oil but also by liver oil from a number of other animals. They
also made comparisons between color indices, found by diluting the liver
oils in petroleum ether, and the growth-promoting power of the oils.

They found that a high color index and a strong growth potency were
parallel.

Thus there seems to be a certain relation between the growth factor and
the chromogenic substance in the liver oil which is the cause of the sulfuric
acid reaction. The authors, however, do not wish to identify these two sub-
stances, although they present several points of conformity. Both
are very sensitive to oxygen and both are thermostable if heated in the
absence of air. Drummond and Watson heated to 100°C. a sample of liver
oil with an especially high color index and strong growth-promoting power,
and passed a current of air through it. At the end of every hour they took
a sample, which was tested for color index and growth-promoting potency.
These factors decreased parallelly and were equal to zero after 5 hours of
heating. The animals which were fed these samples, after being heated for
5 hours or more, developed xerophthalmia, and several of them died from
vitamin A deficiency.

Poulsson and Weidemann (1923) modified the technique of Drummond and
Watson. In order to avoid the carbonization caused by the sulfuric acid,
which they feared might conceal weak reactions, and to obtain a constant
acid concentration, they employed chloroform saturated with sulfuric acid.
They found, as did Drummond and Watson, that a high color index and a
strong activity, as a rule, correspond.

Drummond and Watson tried the reaction in butter fat and found that
it is less potent than in liver oil. This they thought was due to the quantity
of vitamin A being about 250 times less in butter fat than in liver oil (Zilva and Miura, 1921). Poulsson and Wiedemann did not succeed in making butter fat yield a reaction.

I first tried the experiment according to the information given by Poulsson and Weidemann, but in spite of all my attempts to reproduce their experiment I did not succeed in producing the reaction in any kind of liver oil at my disposal. Guided by the statements of Drummond and Watson, I therefore employed petroleum ether as a means of dilution and pure sulfuric acid, sp. gr. 1.84, as a reagent.

**Method.**

The experiment is made with a total quantity of 5 cc. First, the petroleum ether needed is measured out with a pipette, then is added the fatty substance which has, if necessary, been melted in advance by gentle heating on a water bath. From a pipette 1 drop of sulfuric acid is added. The glass is shaken, and the bluish violet-purple color appears quickly and as quickly disappears. After a few minutes a small colored deposit of carbonized organic substance is precipitated.

By this method I examined different samples of liver oil (Table I).

<table>
<thead>
<tr>
<th>Liver oil concentration per cent</th>
<th>Reaction</th>
<th>Remarks.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>1.25</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>0.63</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>0.31</td>
<td>0</td>
<td>Solution faintly opalescent.</td>
</tr>
</tbody>
</table>

 +++ = strong color reaction; ++ = medium strong reaction; + = faint reaction; and 0 = no reaction.

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By this method I examined different samples of liver oil (Table I).

The other two samples of liver oil reacted in the same manner.

**Experiments with Butter Fat.**

The butter fat is of the same sort as that employed at the Institute as a vitamin A-containing substance for the experiments on
rats. It consists of common butter, heated gently on a water bath and kept in a separatory funnel. The water with the separated casein is then tapped off, and the butter fat strained through cotton.

In 1920 Drummond and his collaborators found that lard contained vitamin A if the pigs were fed on greens. Vitamin A was, however, found only in small quantities. These authors further discovered that lard lost its vitamin content if heated in an open pan in the air. Hopkins (1920) and Drummond and Coward (1920) have proved that this is due to oxidation.

**TABLE II.**

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td>cc.</td>
<td>per cent</td>
<td></td>
</tr>
<tr>
<td>1.25</td>
<td>3.75</td>
<td>25</td>
<td>++</td>
</tr>
<tr>
<td>1.00</td>
<td>4.00</td>
<td>20</td>
<td>++</td>
</tr>
<tr>
<td>0.50</td>
<td>4.50</td>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td>0.25</td>
<td>4.75</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>0</td>
<td>4.90</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

**TABLE III.**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td>cc.</td>
<td>per cent</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>2.50</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>1.25</td>
<td>3.75</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>1.00</td>
<td>4.00</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>0.50</td>
<td>4.50</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

I therefore tried to make lard yield a reaction. The lard employed was produced from abdominal fat from a pig, melted out at as low a temperature as possible, and then strained through gauze.

At all the concentrations, no color was apparent, only a faint opalescence.

In 1923 Fridericia recorded some experiments on mice in which it was stated that if the mice were fed on butter fat and hardened whale oil melted together as a vitamin A addition to their fundamental food, the animals died from deficiency of vitamin A. Con-
sequently, the vitamin A of the butter fat must have been destroyed or the whale blubber had a toxic effect on the animals. Later experiments on rats, however, proved (Fridericia, 1924–25) that whale blubber did not act as a toxic agent on the animals, but on the butter fat. The vitamin A deficiency did not occur when butter fat and hardened whale oil were not melted together but given separately, or when the hardened whale oil was kneaded together with the fundamental food and butter fat given separately.

Fridericia examined other hardened fatty substances, coconut oil and hemp-seed oil, in the same manner, but neither of them displayed any destructive power on vitamin A. Later he demonstrated that lard, which had been heated to 102–105°C. during 24 hours on a flat tin tray in the presence of air, when kneaded together with butter fat made this latter ineffective as a vitamin A-supplementing factor. Lard not having been heated in this way was devoid of this power.

I made a mixture of equal parts of the previously tested butter fat, which yielded a positive sulfuric acid reaction, and lard, which had been heated to 102°C. during 24 hours.

After 10 minutes, the time needed for the lard to act on the butter fat, the experiment was started.

A few seconds after the sulfuric acid had been added, but later than the purple color generally appears, the solution became brown, the intensity varying according to the concentration. At a lower concentration a brown deposit appears after a lapse of about 18 hours, while the upper part of the solution is colorless. I repeated the experiment several times; each time obtaining the same result.

I next tried a mixture of butter fat and lard, produced in the same manner, but not having been heated.

<table>
<thead>
<tr>
<th>Fatty mixture</th>
<th>Petroleum ether</th>
<th>Mixture</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 cc.</td>
<td>2.5 cc.</td>
<td>50 per cent</td>
<td>0</td>
</tr>
<tr>
<td>1.25 cc.</td>
<td>3.75 cc.</td>
<td>25 per cent</td>
<td>0</td>
</tr>
<tr>
<td>1.00 cc.</td>
<td>4.00 cc.</td>
<td>20 per cent</td>
<td>0</td>
</tr>
<tr>
<td>0.50 cc.</td>
<td>4.50 cc.</td>
<td>10 per cent</td>
<td>0</td>
</tr>
</tbody>
</table>
As it may be of interest to know the proportions of the mixture of butter fat and heated lard not yielding a sulfuric acid reaction, the following experiments were performed.

With a mixture of 20 per cent of heated lard and 80 per cent of butter fat, the reaction is weakly positive. With a lesser proportion of lard, it is distinctly positive.

Furthermore, it appeared that, even if the mixture of the two substances is made directly in the test-tube and the experiment immediately undertaken, the result is the same as that shown in Table IV.

In order to find out whether the reaction would disappear by heating the butter fat, the following experiment was made.

A sample of the previously positively reacting butter fat was heated on a flat tin tray for about 4 hours to about 100°C. in the presence of air.

The same brown color appeared in this experiment as when lard was heated and when butter fat and heated lard were mixed; but never an indication of the purple color.
DISCUSSION.

From my experiments and those of other investigators, it appears that butter fat gives a reaction with sulfuric acid and, compared with liver oil, a stronger one than ordinarily recorded. In this connection it certainly is of consequence that the butter fat has been derived from grass butter (Drummond, Coward, and Watson, 1921), whereas the samples of oil, employed by me, had been left standing for a long time, with the result that the chromogenic substance had decreased.

It also turns out that when butter fat is mixed with lard which had been oxidized by heating to 100–102°C. in the air during 24 hours, its power of yielding a sulfuric acid reaction decreases at the same time as the growth-promoting potency disappears. It is to be supposed that substances arise in the lard capable of destroying vitamin A as well as the chromogenic substance in the butter fat, and that these destructive agents are probably peroxides (Fridericia, 1924–25). It is at any rate most probable that the destruction is effected by oxidation, and as a mixture of butter fat and non-heated lard does not prevent the reaction, the destructive substance must be due to the heating on exposure to the air.

Further, it appears from Table VI that the action is quantitative. If the quantity of heated lard does not surpass 20 per cent of the total quantity of fatty substance, the reaction is yielded, although much less pronounced than in pure butter fat or in a mixture of butter fat and non-heated lard. Whether this is altered when the mixture is left standing in order that the fatty substance may have more time to act upon the chromogenic substance of the butter fat, I have not yet examined. It is, however, hardly probable, as an experiment of mixing butter fat and heated lard in a test-tube and directly performing a test for sulfuric acid proved the destruction is instantaneous.

As may be seen, my experiments, as many other researches, serve to demonstrate that there exists an intimate association between the chromogenic substance and the growth-promoting power. They also show that as the growth-promoting power disappears in a mixture with heated lard, so the ability for a typical reaction with sulfuric acid likewise disappears.
SUMMARY.

1. Butter fat gives a reaction with sulfuric acid but not in such low concentrations as cod liver oil, and color indices do not attain so high a value.

2. The lard examined does not give a reaction with sulfuric acid.

3. A mixture of equal parts of butter fat and non-heated lard gives a reaction with sulfuric acid.

4. When lard is heated to 100–102°C. during 24 hours in the air and then mixed with lard, the mixture does not give a reaction with sulfuric acid. The growth-promoting power disappears simultaneously, as shown by Fridericia (1924–25).

5. If the heated lard amounts to less than 20 per cent of the mixture, the only result is a decrease in the intensity of the color index at the reaction with sulfuric acid.

6. When butter fat is heated 4 hours in the air its ability for a typical sulfuric acid reaction disappears.

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