THE EFFECT OF FEEDING SUGAR UPON THE ESTERASE CONTENT OF THE BLOOD SERUM AND ORGANS IN PHOSPHORUS POISONING.

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The so called sparing action of carbohydrates upon protein metabolism has long been known. The mechanism of the process is probably closely related to the protective effect of a carbohydrate diet upon certain types of poisoning believed to be associated with increased protein metabolism. Opie and Alford\(^1\) demonstrated that the feeding of starches and sugars in large amounts renders experimental animals (rats) less susceptible to poisoning by chloroform and phosphorus. Graham\(^2\) showed that the animals whose livers contained large amounts of glycogen suffered less readily from delayed chloroform poisoning, even when the anesthesia was continued for considerable periods of time. A series of experiments carried out in this laboratory in 1915-16 fully confirmed these results. It seemed probable that this protective action of carbohydrates was in some way dependent upon the intracellular enzymes. This report presents the results of a study, made at that time, of the effects of feeding sugar and of poisoning by phosphorus and chloroform upon the esterase content of the blood serum and of extracts of the liver, spleen, and kidneys.

Interest in the lipolytic activity of blood serum and tissues dates from 1896 when Hanriot\(^3\) demonstrated the presence in blood serum and in extracts of liver and pancreas of a ferment capable of hydrolyzing fats and oils. Hanriot also

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\(^3\) Hanriot, M., Compt. rend. Acad., 1896, cxviii, 753, 833.
used monobutyrin as substrate. He called the ferment "lipase." In 1900 Kastle and Loevenhart, using ethyl butyrate as substrate, proved the presence of lipase (esterase) in a number of organs and tissues, notably in the liver, stomach, and small intestine. Loevenhart made further studies and found this enzyme in varying amounts in all tissues tested, being present in greatest abundance in the locations in which fat synthesis is known to take place. Quinan studied the ester-splitting ferments of the liver, kidney, and muscle of guinea pigs, and concluded that the enzyme concentration per gm. of tissue is characteristic of, and constant for each organ. He also suggested that the quantitative relations between the lipase (esterase) content of different organs and tissues may be proportional to their cellularity.

The esterolytic power of the blood serum in various pathologic conditions arising spontaneously and produced experimentally has been studied by a number of investigators. Whipple, Jobling, Eggstein, and Petersen, and Sagal observed an increase in the serum esterase in the pathologic conditions associated with destruction of liver substance, such as phosphorus and chloroform poisoning.

The concentration of esterase in diseased tissues has not been so extensively studied. Winternitz and Meloy found that when microscopic fat was present in the liver, the esterolytic activity was decreased. The diminution was not, however, proportional to the amount of visible fat present. The esterase content of the kidneys varied greatly in nephritis. Quinan, working with guinea pigs, found that the loss of esterolytic ferment, per gm. of liver, after prolonged intoxication with chloroform may be as great as 38 per cent. Quinan suggests that chloroform disturbs the "lipase balance," because he observed a decrease in the amount of that enzyme in the liver and a corresponding increase in the kidneys and muscle. Jobling, Eggstein, and Petersen found that liver tissue showing fatty degeneration, obtained from animals poisoned with phosphorus or chloroform contained a decreased amount of esterase.

Loevenhart, on the other hand, states that the fatty changes occurring in phosphorus poisoning are not due to changes in the amount of esterase in the tissues, as no disturbances of this character were noted. Ducceschi and Almagia found no changes in the ester-splitting power of the livers of animals poisoned.

with phosphorus. Saxl\textsuperscript{13} did not note any increase in the esterase of the liver in phosphorus poisoning.

The methods used by different investigators to prepare their extracts of organs have varied widely. Kastle and Loevenhart\textsuperscript{4} in a part of their work, Quinan,\textsuperscript{5} and others have ground up weighed quantities of the organs studied, with or without the use of sand, and have allowed the ester to come into contact with the tissue fragments. Saxl has called attention to the sources of error in this method. If the reagents remain in contact for only an hour or so the amount of acid produced from the hydrolysis of the ester is too small to be measured with accuracy. If the mixture remains in the incubator for 24 hours the acid produced by autolysis will be a source of error that cannot be neglected. Winternitz and Meloy,\textsuperscript{10} Loevenhart,\textsuperscript{14} and others have used clear filtrates prepared in various ways.

\textit{Technique.}

In the experiments here reported dogs were used. The animals were anesthetized with ether and exsanguinated by opening the carotid artery. As much blood as possible was permitted to flow from the severed vessel by lowering the head and gently massaging the abdomen. The liver, spleen, and kidneys were removed at once. The capsule was separated from the kidney and the fat removed from its pelvis. The whole of each organ was then ground very fine in a meat chopper.

5 gm. of the ground tissue were weighed accurately (to 0.01 gm.) in a wide mouthed bottle. To this were added 10 cc. of glycerol (weighed, not measured). The contents were thoroughly mixed, and the bottle was tightly corked and kept in a dark place at room temperature with frequent shaking for 21 days.

After extraction in glycerol for this period 5 cc. of distilled water were added to the contents of the bottle and mixed well with a stirring rod. The mixture was filtered through a thin pad of slightly moistened absorbent cotton laid on gauze. This gauze-cotton filter was then folded over and the remaining fluid pressed out. The turbid fluid thus obtained was diluted with an equal amount of distilled water and filtered through paper until clear. 1 cc. of the filtrate was, therefore, equivalent to approximately \( \frac{1}{5} \) gm. of tissue. These dilu-

\textsuperscript{13} Saxl, P., \textit{Biochem. Z.}, 1908, xii, 343.
\textsuperscript{14} Loevenhart, \textit{J. Biol. Chem.}, 1906-07, ii, 427.
tions were arbitrary, but were about the minimum that would permit satisfactory filtration through paper. The same technique was employed throughout the series of experiments. The results are therefore comparable.

The esterase content of these extracts was determined as follows: To 4 cc. of distilled water in a test-tube, 1 cc. of tissue extract, 0.25 cc. of ethyl acetate, and one drop of alcoholic solution of phenolphthalein were added. Each tube was shaken until the ester was dissolved and the mixture immediately brought to the neutral point with 0.1 N sodium hydroxide solution. 1 cc. of toluene was then added and each tube shaken forty times. They were placed in the incubator for 24 hours, and then titrated with 0.1 N sodium hydroxide solution. The titrations were all made in duplicate, and those showing differences greater than 0.20 cc. of 0.1 N sodium hydroxide solution were excluded.

EXPERIMENTAL.

As controls, extracts were made from the organs of normal dogs which had not been submitted to any experimental procedures. They were killed by exsanguination under ether anesthesia. The results are shown in Table I.

A number of dogs were submitted to prolonged anesthesia by chloroform. The animal's head was placed in a loosely fitting round test-tube basket which had been covered with gauze. An abundant supply of air was thus assured. The chloroform was allowed to fall drop by drop from a separatory funnel upon the gauze. The anesthesia was continued for at least 4 hours. An average of 200 cc. of chloroform was used for each dog. 2 days later the animals were killed by exsanguination under ether anesthesia. The organs were ground up and extracted in glycerol for 21 days as described above. The esterolytic power of the blood serum and of the organ extracts of two animals, typical of the series, is shown in Table I.

Another series of dogs was given subcutaneous injections of phosphorus in olive oil. They were divided into three groups. Group I received fatal doses of phosphorus. Group II was given large amounts of sugar by stomach tube for 3 days before, and for 1 or 2 days after the injection of the phosphorus. Group III was treated in the same
manner as Group II but the animals were allowed to live for 6 weeks
and were then killed in the manner described above. The protocols
of these animals follow.

Group I.

Dog Ia.—Weight 25 pounds. 27.5 mg. of phosphorus in oil over a period of
10 days. 2 days after the last dose the animal was very ill and was killed by
exsanguination under ether.

Autopsy.—All the tissues were markedly bile-stained. Sections of the liver
showed marked fatty degeneration with necrosis.

Dog Ib.—Weight 22 pounds. 15 mg. of phosphorus over a period of 4 days.
3 days later animal found dead.

Autopsy.—The same lesions were found as in Dog Ia, but they were less marked.

Dog Ic.—Weight 18 pounds. 15 mg. of phosphorus in a single dose. 4 days
later animal extremely ill. Exsanguinated under ether.

Autopsy.—Liver yellow and soft. Sections showed marked fatty degeneration
and necrosis. Hemorrhage into retroperitoneal tissues and into intestinal mucosa.
Blood did not clot in 24 hours. Serum bile-stained.

Dog Id.—Weight 20 pounds. 20 mg. of phosphorus in two equal doses 3 days
apart. 2 days later animal quite ill; killed under ether.

Autopsy.—Liver yellow and soft. Sections showed marked fatty degeneration
and necrosis. No hemorrhages.

Group II.

Dog IIa.—Weight 14 pounds. 150 gm. of sugar daily for 3 days, then 15 mg.
of phosphorus in a single dose. 150 gm. of sugar on following day. 3 days later
animal ill with cough.

Autopsy.—Lesions of distemper. Liver yellow. Sections show much glycogen
and only a small amount of fat in the liver.

Dog IIb.—Weight 20 pounds. 150 gm. of sugar daily for 3 days. 15 mg. of
phosphorus in a single dose. 150 gm. of sugar on following day. Animal remained
in excellent condition; very lively. Killed on 4th day after injection of
phosphorus.

Autopsy.—Liver yellowish. Small hemorrhage in retroperitoneal tissues about
pancreas. Blood did not clot in 24 hours. Sections showed glycogen and some
fat in the liver.

Dog IIc.—Weight 23 pounds. Jan. 4 to 6, 1916, inclusive, 300 gm. of sugar
daily. Jan. 6. 15 mg. of phosphorus. Jan. 7 and 8, 150 gm., and Jan. 9 and 10,
300 gm. of sugar daily. Jan. 9. 10 mg. of phosphorus. Animal remained in
excellent condition; very lively. Jan. 11. Killed under ether.

Autopsy.—Liver yellowish. No hemorrhages. Sections show large amounts
of glycogen and relatively little fat in the liver cells.
Dog IIId.—Weight 21 pounds. Treatment identical with that of Dog IIc. Killed under ether.

Autopsy.—Liver yellowish. Some hemorrhage about the right half of the pancreas. Sections show considerable glycogen and some fatty degeneration of the liver.

Group III.


Dog IIIb.—Weight 22 pounds. Treatment like that of Dog IIIa except that 20 mg. of phosphorus were injected on Dec. 20. Feb. 9, 1916. Killed under ether.

The esterase content of the blood serum and of the organs of these animals poisoned by phosphorus is shown in Table I.

A second group of control animals was fed large amounts of sugar in 40 per cent solution by stomach tube for a period of 4 to 6 days, and killed under ether anesthesia on the day following the last feeding. The livers of all of these animals were definitely yellowish in color, the degree of coloration varying roughly with the quantity of sugar fed. All showed large amounts of glycogen in the liver cells. The esterolytic activity is shown in Table I.

DISCUSSION.

It is seen from Table I that the esterase content of the serum and livers of normal dogs is reasonably constant. The weakest liver extract required 3.20 cc., and the strongest, 3.45 cc. of 0.1 N sodium hydroxide to neutralize the acid produced. The average was 3.30 cc. The esterolytic power of the extract of the spleen was less than one-tenth that of the liver. This is not in harmony with the statement of Fiessinger and Marie\textsuperscript{15} and of Bergel\textsuperscript{16} that lymphocytes are especially rich in esterase. The esterase content of the kidneys of the control animals was very variable. Winternitz and Meloy\textsuperscript{10} found marked variations in the esterase of human kidneys. The change from the normal, usually a decrease, observed by them in


\textsuperscript{16} Bergel, S., \textit{Münch. med. Woch.}, 1909, lvi, 64.
nephritis was not proportional to the degree of involvement of the organ. Nephritis is not uncommon in street dogs, and a number of the animals used in these experiments showed more or less severe lesions of the kidneys. This may account for the irregularity of the results. The figures obtained for the normal animals vary too greatly

TABLE I.
Esterase Content of Blood Serum, Liver, Spleen, and Kidney Expressed in Cubic Centimeters of 0.1 N Sodium Hydroxide Required to Neutralize the Acid Produced from Ethyl Acetate.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Blood Serum</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; 2</td>
<td>0.25</td>
<td>3.25</td>
<td>0.30</td>
<td>1.15</td>
</tr>
<tr>
<td>&quot; 3</td>
<td>0.30</td>
<td>3.45</td>
<td>0.20</td>
<td>0.60</td>
</tr>
<tr>
<td>&quot; 4</td>
<td>0.40</td>
<td>3.20</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Chloroform 1</td>
<td>1.05</td>
<td>3.00</td>
<td>0.20</td>
<td>2.55</td>
</tr>
<tr>
<td>&quot; 2</td>
<td>1.95</td>
<td>3.40</td>
<td>0.35</td>
<td>2.95</td>
</tr>
<tr>
<td>Phosphorus poisoning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I, phosphorus only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ia.</td>
<td>0.85</td>
<td>2.90</td>
<td>0.35</td>
<td>0.85</td>
</tr>
<tr>
<td>Ib.</td>
<td>1.10</td>
<td>3.35</td>
<td>0.85</td>
<td>2.25</td>
</tr>
<tr>
<td>Ic.</td>
<td>0.50</td>
<td>3.90</td>
<td>0.35</td>
<td>2.80</td>
</tr>
<tr>
<td>Id.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II, phosphorus and sugar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIa.</td>
<td>1.15</td>
<td>4.40</td>
<td>0.30</td>
<td>0.80</td>
</tr>
<tr>
<td>IIB.</td>
<td>0.60</td>
<td>4.15</td>
<td>0.25</td>
<td>3.75</td>
</tr>
<tr>
<td>IIC.</td>
<td>1.55</td>
<td>4.30</td>
<td>0.35</td>
<td>1.40</td>
</tr>
<tr>
<td>IID.</td>
<td>1.05</td>
<td>3.75</td>
<td></td>
<td>2.70</td>
</tr>
<tr>
<td>Group III, recovered</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIIa.</td>
<td>3.45</td>
<td></td>
<td>0.20</td>
<td>0.50</td>
</tr>
<tr>
<td>IIIb.</td>
<td>3.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar-fed 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; 2</td>
<td>0.35</td>
<td>4.80</td>
<td>0.30</td>
<td>1.70</td>
</tr>
</tbody>
</table>

In the case of the animals kept under prolonged chloroform anesthesia the amount of esterase in the blood serum indicates, as shown by Whipple, a serious lesion of the liver. Upon microscopic examination this organ showed the typical central necrosis of chloroform pois-
The esterase content of the liver and spleen was not materially affected. That of the kidneys was apparently increased, at least the amount of acid produced was greater than the highest amount formed by extracts of the kidneys of any of the control animals. The results obtained with extracts of the livers of these dogs thus agree with those of Loevenhart and of Ducceschi and Almagia, rather than with the results reported by Jobling, Eggstein, and Petersen and by Quinan.

Poisoning with phosphorus likewise did not materially change the esterase content of the liver. In all except one animal there was a slight increase. In Dog Ia there was an apparent diminution. Inasmuch as this was the only animal in the series that showed such a reduction, some other plausible explanation of this single instance was sought for. Loevenhart found that bile salts in 0.2 to 1 per cent solution greatly inhibited the action of clear liver extracts upon aqueous solutions of ethyl acetate and ethyl butyrate. It appeared probable, therefore, that the apparent reduction in the case of Dog Ia might be due merely to an inhibition by the bile present in the extract, for it was observed that the extract of this liver was more deeply bile-stained than was that of any of the others. The following experiment was, accordingly, carried out. To 3 cc. of distilled water in a series of test-tubes there were added 1 cc. of liver extract of “Sugar-fed 2” (the strongest extract of the entire series), 1 cc. of a solution of dog bile to make a dilution as shown in Table II, and 0.25 cc. of ethyl acetate. The mixtures were made neutral with 0.1 N sodium hydroxide, shaken up with toluene, and incubated for 24 hours. They were then titrated with 0.1 N sodium hydroxide. The results are shown in Table II. A similar but slightly less effect was also evident with the extract of the kidney of the same animal (Dog Ia). Hence it appears probable that the reduction in esterase activity of the liver of Dog Ia was only an apparent one and was due to the inhibiting action of the bile present in the extract.

It is interesting to note in this connection that Jobling, Eggstein, and Petersen found that the esterase content of the blood from the hepatic vein was less than that from the portal vein. They concluded from this observation that the increased esterase content of the blood serum in phosphorus poisoning does not come from the destroyed...
liver cells. In view of the results recorded in Table II it is possible
that the greater concentration of bile in the blood of the hepatic
vein may account for the difference observed by these authors. The
question whether in jaundice the bile leaves the liver by way of the
lymphatics or is absorbed directly into the blood is still a matter of
dispute. But the statement of Sabin17 that lymphatics have never
been demonstrated in the adult liver beyond the capsule and the
connective tissue trabeculae strongly favors the view that the bile in
jaundice is absorbed directly into the blood. In that case the con-
centration of bile in the blood of the hepatic vein would be greater
than the concentration in any other part of the circulation.

The feeding of sugar and the consequent storing of glycogen in the
liver is accompanied by a pronounced increase in the esterolytic ac-

<table>
<thead>
<tr>
<th>Dilution of bile</th>
<th>0.1 N sodium hydroxide (ct.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:100</td>
<td>0.85</td>
</tr>
<tr>
<td>1:200</td>
<td>2.80</td>
</tr>
<tr>
<td>1:400</td>
<td>3.75</td>
</tr>
<tr>
<td>1:800</td>
<td>4.20</td>
</tr>
<tr>
<td>1:1,600</td>
<td>4.45</td>
</tr>
<tr>
<td>Control (no bile)</td>
<td>4.80</td>
</tr>
</tbody>
</table>

The feeding of sugar and the consequent storing of glycogen in the
liver is accompanied by a pronounced increase in the esterolytic ac-
tivity of the liver. This effect is not so evident in the other organs
studied, none of which normally stores glycogen. The increase in
esterase is also seen in the animals which were fed sugar before and
after poisoning with phosphorus. Saikowsky,18 Rosenbaum,19 and
Rettig20 have noted the rapid and complete disappearance of glycogen
from the liver in phosphorus poisoning. There is a coincident marked
increase in the amount of visible fat.

The protective action of the feeding of sugar manifested itself both
clinically and histologically. The animals which were given only

18 Saikowsky, Virchows Arch. path. Anat., 1865, xxxiv, 73.
20 Rettig, H., Arch. exp. Path. u. Pharmakol., 1914, lxxvi, 345.
phosphorus became exceedingly ill, and several died as a result of the poisoning. The livers of all of them showed absence of glycogen, marked increase of visible fat, and necrosis. The dogs which were given an equivalent amount of phosphorus with feeding of sugar showed little or no evidence of illness clinically and were always playful. None died as a result of the poisoning. The livers showed glycogen still present, and some visible fat, but no necrosis.

The question as to the identity of the ferment which hydrolyzes the neutral fats (lipase) and that which splits the simple esters like ethyl acetate (esterase) is not yet positively settled. But it seems probable that the relatively small amount of visible fat present in the livers of the animals which were fed on sugar before and after poisoning with phosphorus is in some way closely related to the marked increase in the esterase content of the liver noted in these experiments. It should be observed that the feeding of sugar does not prevent the increase in serum esterase in phosphorus poisoning.

Animals which have entirely recovered from phosphorus poisoning after feeding sugar, still show for some time a slight increase in the esterase content of the liver.

SUMMARY.

2. The esterase content of extracts of the liver and spleen of normal dogs is reasonably constant.
3. The amount of esterase in the liver does not appear to vary to any great extent from the normal, in poisoning with chloroform and phosphorus.
4. Feeding large quantities of sugar and increasing the amount of glycogen in the liver is accompanied by a marked increase in the esterase content of that organ. This increase is also evident in phosphorus-poisoned animals which have been fed large amounts of sugar.
5. The feeding of sugar does not prevent the increase in esterase in the blood serum of animals poisoned with phosphorus.
6. The esterolytic power of extracts of the kidney varies considerably in different dogs.