residue obtained by evaporation of the solvent had the following constants: d_4^{18} 0.960; n_D^{20} 1.500, which approximates the constants found for carvone.

A sesquiterpene was found in the fraction distilling from 260–275° and seemed to be identical with the sesquiterpene found in the wood,[1] though in a state of greater purity. It gave the same color reaction with sulfuric acid in acetic acid solution, and the same amorphous yellow mass on treatment with nitric acid. The constants for this are: d_4^{18} 0.9335; n_D^{20} 1.5039; dextro rotatory. These, while differing from the constants for the sesquiterpene found in the wood, are in accordance with those for a tricyclic sesquiterpene possessing a single double bond.

There was a fraction between the pinene and the limonene fractions which gave the brown flakes with Beckmann's chromic acid reagent, indicating the presence of a pseudo terpene, but no effort was made to place this body definitly, as this investigation was commenced in order to find the aldehyde previously found in the wood, and in this, the investigation was not successful.

A preliminary examination of the oil from the cypress leaves shows this oil to differ widely from the cone oil, and the investigation of the leaf-oil will be undertaken in the near future.

Summary.

The oil from the southern cypress approximates the following composition: Dextro pinene, 85%; dextro limonene, 5%; a pseudo terpene alcohol (sabinol?), 2%; carvone, 3%; a tricyclic sesquiterpene, 3%; the remainder composed of substances boiling above 275°.

No aldehydes were found in the oil.

LOUISIANA STATE UNIVERSITY,
BATON ROUGE, LA.

[CONTRIBUTION FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY OF THE UNIVERSITY OF ILLINOIS.]

FASTING STUDIES. X. A NOTE ON A GLYCOGEN-FREE LIVER.

BY P. B. HAWK.

Received March 27, 1912.

The presence of sugar in the liver was first noted by Claude Bernard,[2] who later demonstrated that glycogen was the source of the sugar.[3] From that day to the present time the nutritional relationships of this polysaccharide have been the subject of a very large number of investigations.[4] The result has been that the origin and function of glycogen in the animal organism are now thoroughly understood.

[1] *Loc. cit.*

[2] Bernard and Barreswil, *Compt. rend.*, **27**, 514 (1848).

[3] Bernard, *Gaz. Médicale*, March 28th, 1857.

[4] For excellent reviews of the literature see Pflüger's "Das Glykogen," Bonn, 1905, and Cremer's review in *Ergebnisse der Physiologie*, **1**, 803 (1902).

It was early noted that the glycogen store of the liver and that of most of the other organs and tissues was lowered during fasting. The heart, however, has been shown to retain its glycogen content unaltered during a period of fasting long enough to decrease the glycogen store of the muscles, for example, to 4–10% of the normal. It was concluded on the basis of early experiments that the liver of an animal could be rendered "glycogen-free" provided the animal in question were subjected to a sufficiently protracted period of inanition. Later experiments cast doubt upon this finding and at present scientific workers quite generally hold to the belief that it is impossible to render the liver "glycogen-free" by means of the fasting procedure. In this connection Pflüger[1] has shown that glycogen could be demonstrated in the liver of a dog after the animal had fasted seventy-three days. In certain recently published tests from our laboratory on postanesthetic glycosuria,[2] data were obtained which were interpreted as indicating the gradual depletion of the glycogen store under the influence of fasting. It was there shown that fasting dogs gave evidence of a progressively decreasing glycosuria following their anesthetization by ether at intervals during the progress of a fast.

It is believed that the glycogen store of the animal body is made up in large part from the transformation of ingested carbohydrates but that there is nevertheless an actual formation continually of small quantities of glycogen within the body of the animal. On this basis, therefore, the hypothesis, that it is impossible to cause the liver of a living animal to give a negative test for glycogen, appears logical. The study of the nutritional relationship of glycogen has been given considerable stimulus through the admirable respiration experiments made by Benedict[3] and collaborators in which the actual course of the glycogen combustion was carefully followed.

In connection with one of our fasting studies, we had the opportunity of examining the liver of "Oscar," our fasting dog, an animal which holds the distinction of having fasted for periods of one hundred and seventeen[4] and one hundred and four days,[5] respectively. The possibility of finding no glycogen present in the liver of this animal had not suggested itself to us. We knew that the glycogen reserve would be depleted in a most pronounced manner because of the great length of the fast (104 days) and we considered that a quantitative determination of the glycogen in this liver would give us some basis for concluding as to the lower limit

[1] Pflüger, *Arch. ges. Physiol.*, 119, 119 (1907).

[2] Hawk, *Arch. Int. Med.*, 8, 39 (1911).

[3] Benedict, Carnegie Publication No. 77.

[4] Howe, Mattill and Hawk, *J. Biol. Chem.*, 11, 103 (1912).

[5] Howe and Hawk reported before Am. Physiol. Soc., Baltimore, 1911; proceedings, *Am. J. Physiol.*, 1912.

for the glycogen reserve as influenced by fasting. Pflüger's method[1] for the quantitative determination of glycogen was employed. *No glycogen was present.* A portion of muscle subjected to the same method also yielded negative results.

To our mind, the unusual length of the fast is associated in a very important manner with the finding of a "glycogen-free" liver. May it not be possible that the nutritional régime of a dog hardy enough to withstand a fast of 100 days will become so altered during the closing days of such a test as to render the organs and tissues glycogen-free? The dog was also a very active animal and delighted to jump in and out of his cage,[2] and, therefore, inasmuch as muscular work will increase the glycogen consumption it is possible that this factor had some slight influence upon the final result. It should also be borne in mind that Oscar was a "repeated" faster. It is possible that the liver of a "repeated faster" subjected to fasts of a very prolonged nature may be rendered glycogen-free, whereas glycogen will remain present in the liver of an "initial faster" through a single protracted fast.

STUDIES ON ENZYME ACTION. II. THE HYDROLYTIC ACTION OF SOME AMINO ACIDS AND POLYPEPTIDES ON CERTAIN ESTERS.

BY K. GEORGE FALK AND J. M. NELSON.

Received April 13, 1912.

1. Introduction.

Very little is known at the present time of the chemical nature of lipase. Differences in the substances which occur in lipase preparations obtained from animal or vegetable sources greatly complicate the investigation into the identity of their active constituents. It is therefore preferable to speak with Loevenhart[3] of the hydrolytic action of enzymes as a definit property without assuming that this property is limited to a single substance or group of substances, until more chemical evidence of the nature of these substances is obtained. Loevenhart, in the paper referred to, discussed in detail the question "*Are the animal enzymes concerned in the hydrolysis of various esters identical?*" and concluded tentatively that while the action of the liver and the pancreas is probably to be attributed to a different single enzyme in each of these tissues, the variations in the actions on different esters by each enzyme are due to variations in the

[1] Pflüger, *Loc. cit.*

[2] Howe, Mattill and Hawk, *Loc. cit.*

[3] *J. Biol. Chem.*, **2**, 427 (1907).