produced an increase in free morphine excretion, also holds true when carbon tetrachloride was used as the damaging agent. In addition to the marked increase in the free morphine there is a decrease in the excretion of the easily hydrolyzable fraction of the combined morphine. As the total recoverable morphine is not materially altered and the difficultly hydrolyzable portion remains fairly constant, the easily hydrolyzed compound appears as free morphine.

One might interpret from these experiments that the easily hydrolyzed form is the only form of conjugation that takes place in the liver, and the formation of the difficultly hydrolyzable fraction occurs at other sites. However, until the conjugation products are identified and their metabolism studied, it is impossible to make any definite statements as to the site of the morphine conjugation. However, liver damage does inhibit the conjugation of morphine to a certain extent as shown by the fact that the free morphine found in the urine is increased without increasing the total morphine excreted.

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## Relation of Pancreatic Secretion to Fatty Changes in the Liver.*

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The question whether following ligation of all pancreatic ducts infiltration of the liver can be prevented by pancreatic juice or by an internal secretion of the pancreas is still controversial.

Dragstedt and collaborators did not observe fatty livers following ligation of all pancreatic ducts, nor following complete loss of all external secretion of the pancreas, and they could not prevent or cure fatty livers by administration of large amounts of pancreatic juice.[1,2] This has been in part confirmed by Boyce and McFetridge,[3] but the results of a number of investigators have been opposed to their findings.[4-10] The latter group found fatty livers following ligation of all pancreatic ducts and Montgomery and collaborators were able to prevent these changes by the feeding of pancreatic juice.[9,10] Our observations in long term experiments on dogs may contribute to decide this controversy.

*Results.* Two animals, with all pancreatic ducts ligated and cut in an aseptic operation and with omentum interposed between duodenum and pancreas were kept for about one year. Both dogs had a good appetite and were kept on a ratio of bread, vegetables, meat and a yeast-bonemeal-salt mixture, with weekly feedings of cod liver oil and dried ox blood. One of the animals lost weight

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slowly and continually, while the weight of the other one did not change materially. Fasting blood sugars were normal. The curves for serum amylase and lipase showed the usual course.[11] Both dogs died 11½ and 12½ months respectively after operation, one with acute enteritis and the other one from trauma and hemorrhage due to a swallowed stone. In both dogs autopsy showed that the pancreas was completely atrophic, appearing as a small, thin, cordlike mass. On microscopic examination it contained scar tissue, many nerve cells and capillaries, an abundance of islets of Langerhans, and very small nests of necrotic acinar tissue. The liver was enlarged and yellow, and on microscopic examination such an extreme degree of fatty infiltration was seen that the characteristic structure was almost obscured.

In 2 other dogs, only the main pancreatic duct was ligated and severed in an aseptic operation and the animals were kept for 8½ and 10½ months respectively on the diet described above. One of these dogs did not lose weight, but the other one showed a marked continuous loss of weight starting 3 months after operation. Autopsy of both dogs showed a pancreas which was completely atrophied with the exception of a piece of normal pancreatic tissue around the small accessory duct, measuring 3 cm in length and 3 mm in width in one dog and being of the size of a peanut in the other dog. Thus only a small portion of the glandular pancreatic tissue had remained functioning. The liver of both these dogs was normal macroscopically and microscopically, with no abnormal fat deposits. The above findings of only a small area of intact pancreatic tissue was exceptional, because in a great number of dogs in whom a similar operation had been performed, much larger parts of the pancreas were found intact after prolonged periods of time.

*Discussions and Conclusions.* Our findings on the appearance of extreme fatty infiltration of the liver in dogs on a mixed diet following severance of all pancreatic ducts contradicts those of Dragstedt and collaborators,[1,2] and of Boyce and McFetridge,[3] and is in accord with those of Berg and Zucker,[4] Aubertin and collaborators,[5] Ralli and collaborators,[6] Person and Glenn,[7] and of Montgomery and collaborators.[8–10]

The last two of our experiments tend to demonstrate that it is the external secretion of the pancreas which, even in small quantities, can prevent the fatty infiltration of the liver cells. While our results confirm those of the second group of investigators mentioned above,[4–10] they do not explain such opposed findings from two groups of reliable workers. We feel that differences in the diets of the experimental animals may be of great importance in the appearance or in the prevention of fatty liver following ligation of all pancreatic ducts. This belief is based on recent findings on the importance of dietary factors, such as proteins, fat and carbohydrate content and balance,[12,13] vitamin $B_1$ content,[14,15] a balance between certain amino-acids,[16–19] a balance between certain proteins and substances with labile methyl

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groups[20,21] and on other nutritional factors not well known as yet.[22-26]

*Summary.* Two dogs with occlusion of all pancreatic ducts showed extensive fatty changes of the liver about 12 months after operation. Two dogs with occlusion of the main pancreatic duct in which only a very small fraction of the pancreas had remained functioning showed no fatty changes of the liver after 7½ and 8½ months respectively. The development of fatty liver is evidently not due to a pancreatic hormone but due to the lack of the external pancreatic secretion and a very small fraction of the normal pancreas and its secretion seems to be sufficient to prevent it.

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## Bacterial Assimilation of Acetic and Succinic Acids Containing Heavy Carbon by *Aerobacter indologenes.**

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Slade *et al.*[1] have shown the fixation of $C^{13}O_2$ in the carboxyl group of acetic acid by *Aerobacter indologenes* and *Clostridium welchii*. The present report provides evidence for the occurrence of the reaction by a cleavage of a $C_4$-dicarboxylic acid. Participation of $C_2$-compounds in the synthesis of 2,3-butylene glycol by *Aerobacter indologenes* is also shown to occur.

Two types of acetic acid were synthesized, (1) $CH_3 \cdot C^{13}OOH$ and (2) $C^{13}H_3 \cdot C^{13}OOH$[2]. Succinic acid ($C^{13}OOH \cdot CH_2 \cdot CH_2 \cdot COOH$) was recovered from various bacterial fermentation. Salts of the acids were added to cell suspensions of *A. indologenes* in the presence of glucose under an atmosphere of nitrogen. The concentration of $C^{13}$ was determined by mass spectrometer analysis. The normal complement of $C^{13}$ is 1.09%.

The distribution of $C^{13}$ on the addition of the acids is shown in Table I. Succinic acid formed in the presence of $CH_3 \cdot C^{13}OOH$ contains $C^{13}$ exclusively in the carboxyl carbons (Table II). Succinate was not formed by a synthesis of pyruvate by $C_1$ and $C_2$ addition, followed by $C_3$ and $C_1$ addition to oxalacetate, for the succinate formed would have contained $C^{13}$ in the methylene carbons. Thus, succinic acid is formed by means of a carbon to carbon linkage involving the carbon† atom originally present in the methyl group of acetic acid. The general reaction is:

$$\begin{matrix} CH_3 \cdot COOH \\ + \\ CH_3 \cdot COOH \end{matrix} \rightleftharpoons \begin{matrix} CH_2 \cdot COOH \\ \cdot \\ CH_2 \cdot COOH \end{matrix} + 2H$$

The reaction is presented with the reservation that acetic acid may or may not be the actual $C_2$ compound involved in the condensation reaction.

Succinate formed in the presence of $C^{13}H_3 \cdot C^{13}OOH$ contains $C^{13}$ equally distributed between the methyl and carboxyl carbons (Table II). This is in agreement with the above reaction and proves that the acetate was not oxidized to $C^{13}O_2$ and the succinate formed by the Wood and Werkman reaction; otherwise the $C^{13}$ would have been found exclusively in the carboxyl carbons.

The formation of $C^{13}$-acetate from succinate demonstrates that the condensation reaction is reversible. There is no mechanism known for the oxidation of succinate by which single carbon atoms may be split off leaving a $C_2$-residue containing a carboxyl carbon of the original succinate.

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