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Histopathological Changes by Acetoacetate in Normal and Scorbutic Guinea Pigs.* (22700)

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Acetoacetate, which on repeated injection has been reported from this Laboratory to induce experimental diabetes by causing inactivation of insulin(1), hyperglycemia accompanied with reduced glucose tolerance(2), depletion of blood GSH(3), depletion of thiamin, riboflavin and niacin(4,5), has recently been found to cause hypovitaminosis C(6), which is also known to bear a close relation with diabetes(7,8). It has also been shown that disturbance in carbohydrate metabolism brought about in scurvy(9) is further aggravated by continued injection of acetoacetate in gradually increasing doses(10). Now because acetoacetate causes depletion of blood ascorbic acid when injected in high doses and histopathology in scurvy has been shown by Mukherjee and Banerjee(11) to reveal widespread degenerative changes in liver, spleen, kidney, testes and suprarenal cortex of chronic and acute scorbutic guinea pigs and since β-hydroxy butyric acid, another member of the ketone bodies has been shown by Nath *et al.*(12) to bring about histological changes in the pancreas, it seemed desirable to study the histopathological changes of different tissues of normal as well as scorbutic guinea pigs injected with acetoacetate in gradually increasing doses.

Methods. Fifty male guinea pigs weighing from 300 g to 400 g were divided into 4 groups, each group containing animals of approximately corresponding weights. The last group of animals served as the control, each animal being fed 5 mg ascorbic acid daily supplementing the recommended scorbutic diet (Group I—Control). Group II animals received diet like that of Group I and plus daily injections of acetoacetate (Sodium salt solution of concentration 200 mg/cc at pH 7.2) intraperitoneally, in increasing amounts. The initial dose was 175 mg/kg, increased by 40 mg/kg every third day so that final dose after 21 days was 415 mg/kg (Group II—Acetoacetate injected). Scurvy was produced in animals of Group III by feeding scorbutic diet as recommended by Banerjee(13). Animals of Group IV were fed the scorbutic diet and injected daily with acetoacetate in dosage similar to that in Group II (Group IV—Scorbutic and superimposed with acetoacetate injection). Animals were housed in individual cages. Paired feeding technic was followed to obviate any results due to inanition. The animals of Group II served as the control for this purpose, as it was seen from a preliminary experiment that the diet intake of this group was found to be the lowest as the experiment advanced. The animals of this group were fed the scorbutic diet *ad libitum* and the amount consumed by each was noted every day. The same amount of diet was fed, to every animal of corresponding weight in all the other groups, on the subsequent day. Just after decapitation different tissues of the animals of each group were removed and fixed in Zenker-formol solution. After dehydration

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and clearing, tissues were embedded in paraffin and 4-6 μ thick sections were cut and stained by haematoxylin and eosin stain. Pancreas were stained by Gomori's Phloxin-Haematoxylin method(14). Photomicrographs of different sections were taken under identical conditions.

Results. Histopathological changes in different groups of animals have been presented separately. Pancreas of the Group II animals (acetoacetate injected for 21 days) show an increase in the area of the islet tissues and the number of β-cells. Group III animals (Scurvy) show distinct early hypertrophy and early degranulative changes of the pancreatic islet cells but no degenerative changes. Group IV (Scurvy superimposed by repeated injection of acetoacetate) show more clear changes. Fibrosis and degranulation are more distinct. Hypertrophy of the islet cells and pyknotic changes in the nuclei, which precede degenerative symptoms of the β-cells are also evident. Animals of this group also show clear evidence of small degree of lipomatosis.

Kidney of Group II animals (acetoacetate injected) show hypertrophy of the glomeruli and early hyalinization of the proximal convoluted tubules. Changes in the tubular cells and nuclei are indicative of hyperfunction. Group III animals (scurvy) show atrophy of the glomeruli and degenerative changes in the tubules. Kidneys of the Group IV animals (scurvy superimposed by repeated injections of acetoacetate) show mild intercapillary glomerulosclerosis. Capsular cells are also distended. Proximal convoluted tubules show the presence of hyaline casts and necrosis.

Liver of the Group II animals (acetoacetate injected) show hydropic distension of the hepatic cells and pyknosis of a few nuclei around the central vein. In Group III animals (scurvy) hepatic cells show distinct signs of early degeneration. In some cases nuclei are found to be pushed to the periphery of the cells. Liver cells of the Group IV animals (scurvy superimposed by repeated injections of acetoacetate) show characteristic necrotic changes in the peripheral zone of the lobule. Hemorrhage of the hepatic cells is also seen around the central vein.

Discussion. Previous studies have shown that repeated injections of acetoacetate to guinea pigs kept on scorbutic diet cause a rapid development of hyperglycemia and depletion of glycogen storage of liver and leg muscle(10). Continued injection of acetoacetate alone can also cause different changes in carbohydrate metabolism similar to that observed in diabetic condition. Scurvy has been known to cause degranulation of the β-cells of the pancreas(15), and to lead to various disturbances in carbohydrate metabolism(9). Repeated injection of acetoacetate has also been found to destroy vit. C *in vivo* (6). The role of acetoacetate in the aggravation of the disturbances of carbohydrate metabolism in scorbutic condition(10) has thus been accounted for.

The present study of the histopathological changes in different tissues of the animals kept on scorbutic diet and simultaneously injected with repeated doses of acetoacetate is in keeping with the biochemical lesions reported earlier(10). Pancreas of the scorbutic animals show early degranulative changes. Acetoacetate injection alone can also cause hypertrophic changes but when both conditions are superimposed, histopathology of the pancreatic islet cells is considerably aggravated showing fatty infiltration and early fibrosis of the tissue and pyknotic changes of the nuclei indicating overactivity and exhaustion. Kidney changes also present similar results. Acetoacetate injection alone causes hypertrophy, showing hyperfunction of the tissue, indicating more work for the cortex to metabolize acetoacetate. Degeneration and atrophy of the tubules begin with scurvy; but scurvy superimposed with the injection of acetoacetate lead to intercapillary glomerulosclerosis a condition which appears commonly in clinical diabetes(16).

Hydropic degeneration of liver cells in acetoacetate treated animals may be due to toxicity of acetoacetate injections. Scorbutic hepatic cells show early degenerative changes, which are aggravated leading to necrosis and haemorrhage around the central vein when the acetoacetate injections are superimposed on scorbutic condition.

All these findings demonstrate the toxicity of acetoacetate injections upon the structural integrity of the tissues. They also confirm and explain the biochemical findings as regards the aggravation of disturbances in scurvy by acetoacetate(10). The accumulative disturbances in carbohydrate metabolism thus produced finally tend to affect the architecture of the pancreatic islets accompanied with the degeneration of the nuclei of the β-cells, commonly observed in clinical diabetes(17), thus confirming the deleterious effect of acetoacetate in inducing diabetes in experimental animals and disclosing a possibility of excess accumulation of acetoacetate serving as a prominent factor for gradual development of clinical diabetes.

Summary. The islets of Langerhans of scorbutic guinea pigs and those injected with acetoacetate were hypertrophied. Injection of acetoacetate into scorbutic animals caused further degranulation and degenerative changes. Acetoacetate brought about hypertrophy of renal cortex and scurvy caused atrophy of glomeruli. Combined treatment aggravated these degenerative changes. Both scurvy and acetoacetate caused central degeneration of liver and this is enhanced by the combined treatment. In agreement with histologic changes, biochemical findings indicate the toxicity of acetoacetate. Acetoacetate may be a prime factor in clinical development of diabetes.

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Purification of Pancreatic and Urinary Callicrein. (22701)

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The hypotensive substance callicrein (kallikrein or padutin) was first discovered by Abelous and Bardier(1) and systematically studied by Frey, Kraut Werle and co-workers for the next 3 decades(2-3). Procedures for the purification of both urinary and pancreatic callicrein(4-10), with the exception of that developed by Reisser(10), depend to some extent on the adsorption of impurities and/or callicrein on various colloidal salts. Since adsorption conditions are difficult to reproduce and frequently require the running of test samples before each step in the preparation,* the present method was devised based on an ethanolic fractionation scheme such as carried out by Cohn *et al.* upon plasma proteins(11) and utilized successfully by this laboratory for purification of hyaluronidases(12-13).

Methods. Callicrein content of the various fractions was determined by the method of

* Werle, E., personal communication.