Studies on Pathogenesis of Atherosclerosis (Report I)

1. The Effects of Hepatotoxic Substances, Gingseng Extract and Whole-body X-irradiation on In Vitro Incorporation of Acetate-1-C^{14} into Cholesterol.

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There are studies indicating a delayed increase in the rate of hepatic biosynthesis of fatty acids in whole-body X-irradiated albino rats, as indicated by measurements of acetate-1-C^{14} incorporation [1~3]. And, in particular, considerable augmentation of cholesterol biosynthesis from acetate in the albino rat liver and adrenals by exposure of the animals to X-irradiation[4,5] and also by injection of the detergent Triton WR 1339[6] have been reported. On the other hand, suppression of the biosynthetic rate was obsevred in animals deprived of food for 24 to 48 hours[7,8] or when fed a cholesterol-enriched diet[9~13] or when administered 4-cholestenone[14,15].

Thus, regulation of cholesterol homeostatis in animals by variation in the rate of cholesterol biosynthesis appears to be primarily a function of the liver. It is, therefore, quite natural to expect that radiant energy absorbed in the liver tissue will no doubt cause a significant disturbance with regard to homeostasis of living cells. In addition to X-ray, the author was interested in any alteration of cholesterol biosynthesis caused by administration of ginseng extract, carbon-tetrachloride and ethyl-alcohol to albino rats.

Though there are many steps not yet elucidated regarding cholesterol biosynthesis from acetate, it is postulated that three molecules of acetate combine to form branched chain intermediates of six carbon, which, in turn, yield by multiple condensationsqualen. Squalen, in a later stage, is cyclized into lanosterol which is, in turn, transformed into cholesterol.

The purpose of the present investigation was to study the variation in the biosynthetic rate of cholesterol in the liver after whole-body X-irradiation and ginseng extract, carbon-tetrachloride and ethyl-alcohol administration to albino rats compared with that in normal rats by measuring the incorporation rate of acetate-1-C^{14} into a cell free homogenate of liver.

Experimental

Materials; Acetate-1-C^{14} was purchased from the Radio-chemical Centre, Amersham, U. K. The solution was prepared by diluting it with physiological saline solution to make its activity 25 uc per mg acet ate. Diphosphopyridine nucleotide and adenosine triphosphate were purchased from the Nutritional Biochemicals Corporation. Cholesterol and digitonin were purified by recrystalization. Ginseng extract was prepared by extracting 10 gm of dried powder of Keumsan product with 100 ml of boiling water for 24 hours. The concentration of the extract(pH 5.9) employed in this experiment was 0.079 mg N/ml, 0.072 mg P/ml and 0.25 mg glucose/ml. Into each incubating flask 0.5 ml of this extract was added. Carbon-tetrachloride was a Merckproduct: and 0.75 ml and 1.5 ml of 20% solution of it were injected intramuscularly into two groups of rats, fasting for 6 hours, which were sacrificed by decapitation 24 hours later. For 8 weeks animals were daily fed by gastric tube 33% ethyl-alcohol(2 ml per 100 g body weight).

Animals; Mixed-bred Wistar rats, regardless of their sex, weighing 100 to 150 gm, were maintained in the rat cage; and selected at random before use. For X-irradiation, rats were exposed to a dose of 600r at a rate of approximately 27 r/min, delivered by 140 KV machine at a distance of 50 cm with 2 mm aluminum filter. The animals in group a were killed by decapitation 24 hours and in group b 6 days after

X-irradiation.

Preparation of tissue; Fresh livers of decapitated animals were removed as quickly as possible and were placed immediately on ice. After the liver was perfused with distilled water to remove blood, it was inserted between filter paper to eliminate fluid. A definite portion of the liver was homogenized in 2.5 volumes of 0.1 M phosphate buffer of pH 7.0, after the method of Singer, et al.[16]. After centrifugation for 10 minutes at 700 x g, 5 ml of the supernatant solution as a source of enzyme, was placed in a 5 ml Erlenmeyer flask for each determination. To each of these enzyme aliquots was added 0.5 mg of DPN(diphosphopyridine nucleotide) and 0.5 mg of ATP(adenosine triphosphate) in 0.1 ml volumes. As the acetate substrate, 25 uc of acetate-1-C^{14} in 0.1 ml was added to each incubating flask.

Incubation; With these contents, enzymatic synthesis of cholesterol was carried out in the flask for 2 hours at 34°C in an oven aerobically with occasional shaking.

Analysis; After incubation, 0.5 mg of cholesterol was added to each flask as the carrier; the entire incubation mixture was subjected to saponification with alcoholic KOH in a boiling water bath. After the saponification process was complete, the content was acidified by addition of small amount of acetic acid. The acidified mixture was extracted with ether and the cholesterol was isolated as the digitonide, which was in turn dissolved in a small amount of absolute alcohol, and evaporated very slowly on an aluminum planchette under an infra-red lamp. Radioactivity counting was carried out on this cholesterol digitonide in a gas flow Geiger-counter, and expressed as counts/minute/planchette (g tissue).

Results and Discussion

In Talbe I are shown the alteration of in vitro enzymatic biosynthesis rate of cholesterol from acetate-1-C^{14} as a substrate by addition of ginseng extract and hepato-toxic agents such as alcohol and CCl_4 and by whole-body X-irradiation.

As is apparent from the results, additions of the ginseng extract, carbon-tetrachloride and alcohol each caused a significant increase of the incorporation rate of acetate-1-C^{14} into cholesterol in vitro. The most drastic increase of the biosynthesis rate of cholesterol from acetate was observed with alcohol administration, while the least was observed with ginseng extract administration.

Ginseng is believed to increase the metabolic rate by accelerating the catabolism of protein, as it promotes not only diuresis but also causes an elevation of the concentration of total nitrogen in the urine[17]. And there is other evidence confirming this when ginseng is administered in small amounts to animals[15~20].

Recently it has been reported that the addition of ginseng extract to liver slices and mitochondria caused an increase of oxygen consumption both in normal and carbon-tetrachloride treated albino rats[21]. In the present study, one of the various physiological actions of ginseng on animal tissue is well illustrated in connection with cholesterol biosynthesis. As seen in Table I, 0.2 ml of ginseng extract increased the incorporation rate of acetate-1-C^{14} into cholesterol approximately 38% and 1.5 ml of it approximately 100%.

However, in the group administered carbon-tetrachloride, the effect was inversely related to the dose administered; that is, with an increased amount of carbon-tetrachloride, a decrease in the incorporation rate was noted, unlike the effects observed with other materials tested. In a and b groups, given carbon-tetrachloride, the incorporation was increased by 163% and 96.4% respectively (Table 1). In fact carbon-tetrachloride is known to exert a hepatotoxic effect and in later stages results in fatty degeneration[22].

On the other hand, it is apparent that alcohol administration was observed to cause the same effects as that of ginseng and X-irradiation. As shown in Table 1, almost five times the normal incorporation rate was observed in the long-term administration of alcohol to rats (b), while three times in the short-term administration of alcohol to rats (a). This intensive increased rate of in vitro cholesterol synthesis following alcohol administration is, therefore, indicative of stimulative or accelerative action of alcohol so far as in vitro acetate-1-C^{14} incorporation into cholesterol is concerned. As a matter of fact, alcohol in an excessive amount interferes considerably with tissue metabolism[23], and in the long run results in fatty infiltration and Laennec's cirrhosis[24,25].

Besides this toxic effect, alcohol per se as a carbo-

Table 1. Radioactivity of Cholesterol Synthesized in Rat Liver in Vitro from Acetate-1-C^{14} (counts/minute/g tissue.)

Control group		Ginseng (10% water extract) added group		CCl_4 (20% olive oil solution) administered group		33% EtOH (2 ml per/g body wt.) administered group		X-irradiated (600 r) group	
		a[1]	b[2]	a[3]	b[4]	a[5]	b[6]	a[7]	b[8]
	99.5§	142.5	228.0	300.5	204.5	267.5	448.5	168.5	253.3
	95.5	142.5	191.5	244.5	203.0	268.0	510.5	105.0	250.0
	99.0							217.0	
	120.5								
Mean	103.6	142.5	209.7	272.5	204.5	277.5	479.5	163.5	252.3
%	100.0	137.5	202.4	263.0	196.4	267.8	462.8	157.9	244.5

§—All figures denote average values of the determinations on 5 albino rats.

1—Addition of 0.2 ml of the ginseng extract in vitro.

2—Addition of 0.5 ml of the ginseng extract in vitro.

3—Animals administered 0.75 ml of CCl_4 solution.

4—Animals administered 1.5 ml of CCl_4 solution.

5—Animals given alcohol one day.

6—Animals given alcohol daily for 8 weeks.

7—Animals sacrificed 24 hours after X-irradiation.

8—Animals sacrificed 6 days after X-irradiation.

hydrate has another role in lipid metablism. It has been shown that glucose is readily converted not only to fatty acids but also to glycerol[26]. It is concluded, therefore, that glucose is broken down in the glycolytic cycle to pyryvate; the oxidative decarboxylation of the latter yields acetate, which then forms the building unit for the synthesis of fatty acid and choleterol.

The various hypotheses of the possible mechanisms by which fatty acids with a chain of an even number of carbon atoms are formed from pyruvate, have been examined Papjak[26]. Taking into consideration the results of the present study and Papjak's conclusion that conversion of carbohydrate into fat results not only in the formation of fatty acids but also in the entire lipid molecule,including cholesterol, it is evident that administration of alcohol causes a significant rise in synthesizing capacity of liver with regard to lipids.

Whle-body X-irradiation of rats was found to cause a marked increase in the incorporation of acetate-1-C^{14} into liver cholesterol, second to alcohol administration. The rate of increase was about 2.6- and 4.6 -fold 24 hours and 8 weeks respectively after exposure to X-irradiation of 600 r(Table 1). This result is in agreement with Gould's report[27].

However, it is presumed that shortly after X-irradiation the biosynthetic capacity of the liver is influenced. Considering the author's results and Bucher's suggestion[28] that the physiological control of cholesterol biosynthesis in rat liver is influenced primarily by a microsome-dependent step in the segment of the biosynthetic pathway,the author postulates that whole-body X-irradiation activates microsomal funct ion at a dose of 600 r, and therefore the increassed incorporation of acetate-1-C^{14} into rat liver cholesterol results.

Summary

The effects of hepatotoxic agents such as CCl_4 and alcohol and of whole-body X-irradiation on hepatic cholesterol synthesis of rats, were studied in this work. The effect of ginseng water extract on cholesterol biosynthesis was also examined in vitro employing a liver preparation of normal rat.

Throughout this experiment, the in vitro incorporation of acetate-1-C^{14} into cholesterol was studied using hepatic cell-free preparations of rats, treated with either CCl_4 or alcohol, or subjected to X-irradiation, and of normal rats as well.

The results obtained ere as follows;

(1) The capacity for in vitro hepatic cholesterol biosynthesis of rats treated with CCl_4 was increased.

(2) The prolonged alcohol administration also caused an increased cholesterol biosynthesis in rat liver.

(3) The ginseng extract accelerated cholesterol biosynthesis in rat liver.

(4) Whole-body X-irradiation increased the hepatic choleseterol biosynthesis in the rat and to a greater degree as long as 8 weeks after X-ray exposure.

國 文 抄 錄

動脈硬化症成因에 關한 硏究(第一報)

Acetate-1-c^{14} 을 利用할時 肝毒性物質, 人蔘抽出液, 및 X-線全身照射가 肝 Cholesterol 生合成에 미치는 影響에 對한 硏究

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Cholesterol代謝는 Atherosclerosis의 pathogenesis 또는 其他와 關聯되어 最近 醫學界에서 非常한 興味가 集中되어 있다는 것은 再言할 必要가 없다. 또 Cholesterol 代謝에 있어 肝이 重要한 役割을 하는 것은 周知의 事實이다. Cholesterol은 Acetate와 같은 簡單한 化學物質에서 여러 階段을 밟지만 生體에서 쉽게 合成이 된다. 特히 生體基礎代謝硏究에 있어 放射能同位元素가 차지하는 重要性은 至大한 것이다. Cholesterol 代謝 硏究에 있어 Acetate-1-C^{14}를 使用하여 適當한 enzyme source (例컨대 肝 homogenate, cell free preparation 等) 存在下에 incubation으로 incorporation test를 하여 여러 興味있는 事實이 밝혀진 것이다.

本實驗에서 豫備實驗으로 acetate-1-C^{14}와 rat 肝을 使用하여 全身 X-ray 照射及 우리 日常生活에 있어 嗜好物로 重要한 位置에 있는 알콜과 hepatotoxic substance로 有名한 CCl_4 及 人蔘水抽液等이 肝의 Chotesterol biosynthesis에 미치는 影響을 調査하여 여기 그 成績을 發表하는 바이다.

1) CCl^4는 쥐肝의 Cholesterol 生體合成을 크게 促進한다. 그러나 CCl^4量이 많아 肝損傷이 甚하였을 때는 Cholesterol biosynthesis 能은 CCl^4量이 적을때 보다 減少된다.

2) 알콜長期投與로 하여 rat 肝의 Chelesterol 生合成은 크게 促進되며 또 投與期間이 길수록 그 生合成能이 더 促進된다.

3) 人蔘 Extract도 肝의 Cholesterol biosynthesis를 促進한다.

4) X-線 全身照射로 肝의 Cholesterol biosynthesis는 相當히 促進되며 또 X-線照射後 時間이 經過할수록 이 傾向은 增大된다.

(追記 : 本硏究는 1960年度 原子力院 硏究補助費에 依하여 完成되었으므로 이에 原子力院에 對하여 深深한 謝意를 表하는 바이다.)

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