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Effects of 24-Hour Starvation on the Lipid Content of Carbon Tetrachloride-Induced Fatty Liver in Mice

24時間 饑餓가 4鹽化炭素(CCl_4)中毒마우스肝臟의 各種脂質含量에 미치는 影響에 關한 硏究

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The hepatotoxic action of carbon tetrachloride with production of rapid fatty changes and a centrilobular necrosis by a single effective administration, and eventual cirrhosis by repeated administrations, has already been known in experimental animals. Studies in this field have mainly aimed at obtaining greater knowledge regarding mechanism(s) by which, in analogy, human liver damage is produced.

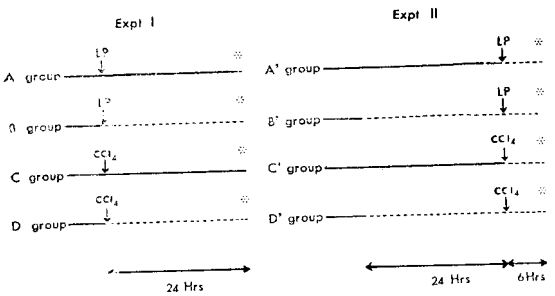
It has also been known for long that when hepatotoxic agents are given to starved animals the injurious effect is increased[1,2,3]. It might be interesting at this time, with the advanced analytical technic, to investigate the effects of starvation on the lipid contents of fatty liver induced by carbon tetrachloride in experimental animal. The importance of such an investigation lies in understanding and comparing many experimental works concerning hepatotoxicity of carbon tetrachloride whether the animal has received food *ad lib* throughout the study or whether food has been withheld for a certain period of time prior to or after the administration of the hepatotoxic agent.

In addition, quantitative observation on the lipids after carbon tetrachloride administration under various conditions, such as intervention of fasting, might help for the elucidation of mechanism(s) involved in induction of fatty liver by carbon tetrachloride.

Effects of a single-facet 24-hour starvation on the lipid content of carbon tetrachloride-induced fatty liver of mice were studied, and will be presented in the following together with the data on carbon tetrachloride-sensitive liver glycogen[4] content determined.

Materials and Methods

Male mice of mixed strain, 11.5-13.0 gm, were used in Experiment I (Expt I), and 12.5-14.0 gm in Experiment II (Expt II). Experimental design in Expts I and II are schematically depicted in Fig 1. Major difference between the two will be found whether the starvation was immediately after (Expt I) or prior to (Expt II) the administration of carbon tetrachloride. In Expt II, in which the 24-hour starvation preceeded the administration of CCl_4 or control substance (liquid paraffin), animals were fasted further, regardless of previous feeding state, following the administration until the time of sacrifice 6 hours later. Amounts of CCl_4 administered intraperitoneally by means of microsyringe, as established by a preliminary test to raise hepatic triglycerides (TG) without lethal



—: fed;
····: fasted;
*: decapitation;
LP: liquid paraffin

Fig 1. Experimental design.

effect in the mice used, was 0.05 m*l* of 3 : 7 CCl_4-liquid paraffin (LP) mixture, which was given to C and D groups in Expt I and C' and D' groups in Expt II. A and B groups in Expt I and A' and B' groups in Expt II were injected 0.05 m*l* of LP only. Throughout the experimental period whether the animals were fed or fasted, *ad lib* access to water was allowed. To stick to strict experimental conditions in subsequent chemical analysis without overloading laboratory burden, paired 8 mice (two for each group in Expt I or Expt II) were treated at a time.

At the scheduled time the liver was removed *in toto* from the animal killed by decapitation, deprived of gall bladder, and weighed on chemical balance (Metler). Equivalent weight of livers from two animals of the same group were pooled in order to secure enough material for the chemical analysis. Lipids were extracted twice in a glass homogenizer by the method of Folch *et al*[5] from accurately weighed fresh liver tissue of approximately 500 mg, with 2 :1 chloroform-methanol mixture to the final volume of 10 m*l* and washed with 2 m*l* of distilled water. After the volume of single phase lipid extract was brought to the 10 m*l* mark in a calibrated test tube with additional chloroform, suitable portions of this extract were used for each fractional liver lipid analysis. TG were determined by the method of Van Handel[6] and Van Handel *et al*[7] with 0.5-1.0 m*l* of washed chloroform-methanol lipid extract following complete evaporation, under mild heat of water bath, and redissolution in chloroform. Commercial olive oil, dissolved in enough chloroform and purified by treatment with bulk amounts of Doucil (W. A. Taylor), the chloroform being evaporated *in vacuo* until the weight of oil became constant, was used as TG standard. Total and unesterified cholesterol were determined by the method of Zak *et al*,[8] with 0.5 m*l* of lipid extract above following its complete evaporation and redissolution in acetone-alcohol mixture for the unesterified form, or 0.3 m*l* of evaporated lipid extract for the total form. Subtraction of the unesterified form from the total would give the value of esterified cholesterol. Phospholipids were determined after the method of Connerty *et al*[9] with 0.5 m*l* of lipid extract directly subjected to acid digestion. For liver glycogen determination approximately 250–1,000 mg of accurately weighed fresh tissue was extracted 3 times with 5% TCA in a glass homogenizer to the final volume of 10–20 m*l* depending on the expected liver glycogen content, and suitable portions were taken to be analyzed according to the method of Carroll *et al* by use of anthrone reagent.[10]

Results

I. Expt I

Effects of 24-hour starvation after the i p injection of CCl_4, according to the experimental scheme (see Fig 1), upon mouse liver weight, and unit weight liver TG, fractional cholesterol, phospholipid and glycogen contents are shown in Table I and visualized in Fig 2 (except

Table I. Liver weight, and triglyceride, fractional cholesterol, phospholipid, and glycogen content after 24-hour fast, intraperitoneal CCl_4, and CCl_4 with ensuing 24-hour fast in mice (Average of 2 pooled livers) (Expt I)

Experiment no. \ Animal groups*	A (Control)	B (Fasted)	C (CCl_4)	D (CCl_4 plus fasting)
1. Liver weight (in mg)				
1	697	620	809	842
2	718	607	815	916
3	708	665	745	766
4	741	620	615	668
5	653	592	785	903
6	701	668	580	878
7	682	623	762	744
Mean	700.0	634.4	730.1	816.7
Standard deviation	27.7	28.5	94.4	92.8
2. Triglyceride content (in mg per gm wet tissue)				
1	5.4	10.9	6.0	47.1
2	10.8	27.5	30.9	163.0
3	9.3	8.8	43.1	56.1
4	8.8	18.1	20.6	68.1
5	4.8	8.4	19.8	35.5
6	8.7	30.2	44.7	59.9
7	8.5	24.4	42.5	67.4
Mean	8.04	18.33	29.66	71.02
Standard deviation	1.83	9.17	14.79	42.04
3. Fractional cholesterol (total, free) content (in mg per gm wet tissue)								
1	5.4	2.9	5.8	2.6	3.9	2.6	9.0	2.7
2	5.8	2.0	6.6	2.3	5.5	2.4	6.8	2.3
3	3.5	1.8	3.7	2.3	3.9	1.9	4.8	2.0
4	2.7	1.9	3.5	1.8	3.3	2.1	5.1	1.4
5	2.3	1.9	4.4	1.8	3.4	1.7	4.5	2.3
6	2.7	1.7	5.7	2.1	3.1	1.4	4.5	2.0
7	3.4	2.1	4.4	1.9	4.6	2.0	5.4	3.1
Mean	3.69	2.04	4.87	2.11	3.96	2.01	5.73	2.26
Standard deviation	1.38	0.40	1.17	0.30	0.84	0.41	1.65	0.54
4. Phospholipid content (in mg per gm wet tissue)				
1	36.0	42.2	31.0	37.8
2	35.6	45.2	29.4	34.8
3	34.2	47.0	28.2	27.2
4	27.0	32.2	31.2	31.2
5	31.6	33.2	29.0	29.8
6	34.4	33.6	21.4	27.6
7	32.4	37.2	29.2	32.4
Mean	33.02	38.66	28.48	37.26
Standard deviation	3.1	6.12	3.26	3.83
5. Glycogen content (mg per 100 gm wet tissue)				
1	2,160	409	385	29
2	2,880	308	148	0
3	1,748	1,179**	36	8
4	4,536	778	24	44
5	3,276	220	215	95
6	2,770	187	18	0
7	2,804	—	69	0
Mean	2,882.0	380.4	127.9	18.0
Standard deviation	891.8	346.2	134.7	40

* For more details on grouping, see text. ** Excluded from the calculation of the mean and standard deviation.

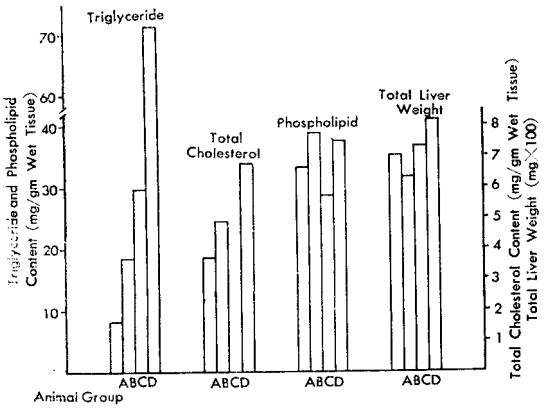


Fig 2. Effects of intraperitoneal carbon tetrachloride with or without ensuing 24-hour fasting on mouse liver triglyceride, total cholesterol and phospholipid contents, and total organ weight.

Animal group A: control; B: fasted; C: CCl_4 administered; D: CCl_4 administered and fasted. (For more details on animal grouping, see text.)

glycogen value). It should be recalled that A group, injected LP and continuously fed up to the time of sacrifice 24 hours later, served as a control with respect to the effect of CCl_4 administration for C group, the animals of which were fed like those of A group but injected with CCl_4. An analogous relationship holds true between B and D groups, both of which were, however, fasted for the final 24 hours after the appropriate injections. Likewise non-fasted A and C groups served as control in respect to starvation for B and D groups, respectively.

1. Liver Weight

In comparison with the mean of 700.0 mg (±27.7, standard deviation) of A group, that of B group showed 634.4 mg (±28.5), a decrease of approximately 10% in total mouse liver weight by simple 24-hour fasting. Compared with that of A group, the mean liver weight of C group, 730.1 mg (±94.4), was an elevation by approximately 30 mg, induced by CCl_4 administration, although it was not statistically significant ($P>0.2$). In fasted animals CCl_4 treatment raised the liver weight from 634.4 mg (±28.5) of B group to 816.7 mg (±92.8) of D group, a remarkable increase. In both groups of CCl_4 treated animals fasting paradoxically raised the liver weight from 730.1 mg (±94.4) of C group to 816.7 mg (±92.8) of D group, which was, however, statistically nonsignificant ($P>0.1$). In summary, simple fasting (B vs A) and CCl_4 administration plus fasting (D vs A) caused significant changes (P's<0.01) toward reduction in the former and elevation in the latter, respectively; whereas simple administration of CCl_4 under fed condition (C vs A) and fasting on top of CCl_4 administration (D vs C) caused nonsignificant changes, both toward elevation of liver weight of experimental animals.

2. TG Content

Effects of CCl_4 and/or fasting as designed in Expt I were most pronounced in unit weight liver TG content than any other lipid parameters measured. Simple fasting (B vs A), simple CCl_4 administration under fed condition (C vs A), fasting upon CCl_4 administration (D vs C), and CCl_4 administration plus fasting (D vs A), all effected consistently significant elevation (P's<0.05–0.01) of unit weight liver TG content in each case cited.

3. Cholesterol Content

Although statistically nonsignificant (except the difference between A and D groups, where $P<0.05$), changes in the unit weight liver total cholesterol content of each group of animals seemed to have more than incidental meanings, *i e*, the highest cholesterol content marked by D group, which possessed the highest liver

Table II. Liver weight, and triglyceride, fractional cholesterol, phospholipid, and glycogen content in 24-hour fasted, intraperitoneally CCl_4-given, and CCl_4-given with previously 24-hour fasted mice* (Average of 2 pooled livers) (Expt II)

Experiment no. \ Animal groups	A' (Control)	B' (Fasted)	C' (CCl_4)	D' (CCl_4 plus fasting)
1. Liver weight (in mg)				
1	955	840	1,297	990
2	823	758	955	1,002
3	802	670	930	650
4	898	749	573	768
5	980	883	490	740
6	935	793	760	728
Mean	898.8	782.2	834.2	813.0
Standard deviation	72.4	47.2	293.3	146
2. Triglyceride content (in mg per gm wet tissue)				
1	25.9	32.4	37.3	127.4
2	15.4	23.8	27.6	34.6
3	6.3	16.4	12.1	14.1
4	5.9	16.8	15.0	46.5
5	6.1	8.8	12.7	20.9
6	4.1	10.7	7.3	32.9
Mean	10.62	18.15	18.67	46.07
Standard deviation	8.49	8.75	11.39	41.42
3. Fractional cholesterol (total, free) content (in mg per gm wet tissue)								
1	3.7	1.8	3.3	1.8	2.8	1.6	4.1	2.4
2	3.2	2.1	3.1	1.8	3.7	1.5	3.4	1.7
3	3.2	1.7	3.2	1.5	3.0	1.5	3.0	1.2
4	3.0	1.8	3.4	1.6	3.0	1.8	3.8	1.6
5	3.5	1.8	3.2	2.0	3.2	2.4	4.4	1.5
6	1.9	1.4	3.0	2.8	2.8	1.4	4.1	1.5
Mean	3.08	1.77	3.20	1.92	3.08	1.70	3.70	1.65
Standard deviation	0.63	0.23	0.14	0.47	0.21	0.37	0.52	0.41
4. Phospholipid content (in mg per gm wet tissue)				
1	40.4	37.4	36.6	25.4
2	36.2	32.6	26.8	26.8
3	38.2	40.0	36.0	34.0
4	31.2	38.2	34.8	36.4
5	32.2	33.6	28.4	37.6
6	31.4	35.0	30.4	40.0
Mean	34.94	36.19	32.18	33.38
Standard deviation	3.89	1.56	4.36	5.95
5. Glycogen content (mg per 100 gm wet tissue)				
1	816	412	112	0
2	980	346	88	0
3	268	270	315	310
4	340	236	212	48
5	415	470	—	—
6	370	140	310	18
Mean	531.5	312.3	207.4	75.2
Standard deviation	92.5	121	106.6	132.7

* All animals were sacrificed after 6 hours of fasting on top of experimental conditions set forth. See text for more details on animal grouping.

weight and unit weight liver TG content among the 4 groups, was another expression of the general tendency of neutral lipid accumulation by the exprerimental condition set forth upon this group. However, whereas the apparent specific action of CCl_4 to raise unit weight liver TG as manifested in C group (TG content: 29.66 ±14.79 mg/gm wet tissue) far exceeded the elevation of TG induced by fasting in B group (TG content: 18.33±9.17 mg/gm w t), this was not the case for total cholesterol content, *i e*, fasting (in B group, total cholesterol content: 4.87± 1.17 mg/gm w t) dominated over the action of CCl_4 (in C group, total cholesterol content: 3.96 ±0.84 mg/gm w t) in regard to total cholesterol accumulation in the unit weight liver tissue studied.

The variation in esterified to total cholesterol ratio went generally along with that of total cholesterol.

4. Phospholipid Content

Changes in the unit weight liver phospholipid content were all statistically significant under the experimental conditions such as fasting (B vs A, $P<0.05$), CCl_4 administration upon fed (C vs A, $P<0.05$) or fasted state (D vs C, $P<0.01$), and fasting following CCl_4 administration (D vs A, $P<0.05$). An inverse relationship between liver weight and unit weight liver phospholipid content could be drawn in A, B and C groups, which might well be expected from the inherent role of phospholipids as structural component of tissues in general. In D group, however, the inverse relationship between the two variables was not evident, which might be another expression of synergistic (?) action of CCl_4 and fasting toward accumulation of lipids in general in liver tissue.

5. Glycogen Content

Together with the data in TG the unit weight liver glycogen content showed a considerable deflection from each other group depending on the experimental conditions imposed, although more marked individual variations in the same group of animals were noted.

II. Expt II

Effect of 24-hour starvation immediately prior to the i p injection of CCl_4, according to the experimental design (see Fig 1) upon mouse liver weight, and unit weight liver TG, fractional cholesterol, phospholipid and glycogen contents are shown in Table II, and visualized in Fig 3 (except glycogen value). It should be emphasized

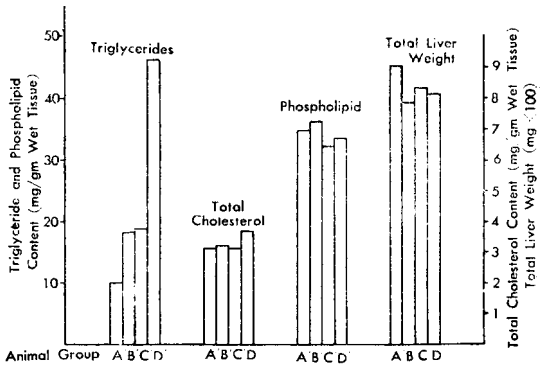


Fig 3. Effects of intraperitoneal carbon tetrachloride with or without previous 24-hour fasting on mouse liver triglyceride, total cholesterol and phospholipid contents, and total organ weight.
Animal group A: control; B; fasted; C: CCl_4 administered; D: CCl_4 administered and fasted (For more details on animal grouping, see text.).

that performance of decapitation took place uniformly in all 4 groups of animals 6 hours of fasting after the treatment of animals with appropriate injections. The group interrelationship from each other in reference with test and control groups was identical with that in Expt I.

1. Liver Weight

Fasted for 24 hours, injected with LP, and sacrificed 6 hours later with further fasting, the mean liver weight of B' group (782.2±47.2 mg) decreased by approximately 12% compared with that of control A' group (898.8±72.4 mg). Previous 24-hour fast effected a nominal decrease ($P>0.1$) on liver weight of CCl_4-given group, *i e*, from 834.2± 293.3 mg of control (C') to 813.0±146 mg of test (D') group. Combined effect of fasting and CCl_4 administration, as manifested in the data of D' group against that of A' group, again resulted in nonsignificant decrease in the liver weight (813.0±146 mg of D' vs 898.8±72.4 mg of A'). These results of slight differences in the liver weight from cach other except that between A' and B' groups were in contrast to the observations made in Expt I.

2. TG Content

Simple CCl_4 administration under fed condition raised 6 hours later TG level (from 10.62±8.49 mg/gm w t of A' to 18.67±11.39 mg/gm w t of C' group, $P>0.1$), but not much more than that by previous induction of 24- plus 6-hour fasting, which caused no significant elevation of TG content (A' of 10.62 ±8.49 mg/gm w t to B' of 18.15 ±8.75 mg/gm w t, $P>0.1$). The difference between B' and D' (effect of CCl_4 within 24-hour fasted groups) or between C' and D' (effect of fasting within CCl_4-given groups) amounted to approximately 25 mg/gm w t in both cases, which, however, were not yet of statistical significance (P's>0.1).

3. Cholesterol Content

The total cholesterol content in unit weight liver of previously fasted and CCl_4-given D' group (3.70±0.52 mg/gm w t) was the highest among 4 groups, with statistical significance when compared with that of A' (3.08±0.63 mg/gm w t) or C' (3.08±0.21 mg/gm w t) (P's<0.05). Since simple fasting (B' vs A') or simple CCl_4 administration under fed (C' vs A') or fasted state (D' vs B') did not affect appreciable total cholesterol content, the change above regarding D' group should be ascribed again, as in Expt I, to the additive effect of fasting and CCl_4 administration (D' vs A') or to the fasting factor alone in CCl_4-given state (D' vs C').

4. Phospholipid Content

None of the experimental conditions set affected any appreciable change in unit weight liver phospholipid content, possibly reflecting minor changes in total liver weight observed. And B' group with the least liver weight had the highest phospholipid concentration, again alluding the inverse relationship between these two variables.

5. Glycogen Content

The decrease in unit weight liver glycogen content from the mean of 531.5 mg (±92.5) /100 gm w t observed in A' to that of 312.3 mg (±121)/100 gm w t in B' group with fasting was without statistical significance ($P>0.2$), whereas the decrease to the mean of 207.4 mg (±106.6) /100 gm w t in C' group by CCl_4 administration under fed state was of significance ($P<0.01$). The apparent glycogen lowering action of CCl_4 was again demonstrated under fasted condition in the data of D' (75.2±132.7 mg/100 gm w t), as compared to that of B' (312.3±121 mg/100 gm w t, $P<0.02$); whereas the reduction from the mean value of C' (207.4±106.6 mg/100 gm w t) to that of D' (75.2±132.7 mg/100 gm w t) induced by fasting alone was without statistical significance ($P>0.1$). It can be deduced from these data that CCl_4 is superior to fasting in depleting liver glycogen content.

Discussion

Treatment of mice with LP or hepatotoxic agent (CCl_4-LP mixture) would reduce the food

consumption by the treated animals compared with non-injected state; yet the discrepancy in such variables as liver weight, TG content, glycogen content, *etc*, between fed A and fasted B, and fed C and fasted D group attested that fed A and C group animals were taking their rations despite the stress incurred by injection. As a matter of fact, the nature of the present work presuppose an active consumption of food by control or test animals as long as foods are available to them. Food consumption as related to injection maneuver was of no concern in Expt II, since feeding (and fasting) preceeded injections and animals were killed all alike after 6 hours of fasting.

Results in previous section stood out in the fact that while many of the data obtained in Expt I bore statistical significance when compared with each other, this did not apply to those obtained in Expt II. The main reason responsible for the relative nonsignificance of data from each other in Expt II, as contrasted to those in Expt I, could be sought, of course, in the different experimental design set upon animals of Expt II from that upon those of Expt I. For instance, the mean liver weight of C group rose to that of D in Expt I (13% increase with statistical significance) by ensuing 24-hour fasting following CCl_4 intoxication, whereas that of C' to D' in Expt II was a contrasting decrease (3% loss with statistical nonsignificance) by 24-hour fasting prior to, and 6 hours after, the injection of CCl_4. Possibly 6-hour fasting after CCl_4 administration complexed with previous 24-hour fast (in D'), as contrasted to simple post-CCl_4 24-hour fast (in D), did not suffice for the stronger action of intraperitoneally injected hepatotoxic agent to reach the liver to call for the accumulation of lipid material and raise the liver weight. The discussion above on the liver weight in relation to time lag after the injection of hepatotoxic agent may also apply to the liver TG content of the animal groups concerned, *i e*, the increase in TG content of D' group (sacrificed 6 hours after the CCl_4 administration with previous 24-hour fast) was less than that of D group (sacrificed with 24-hour fast after CCl_4 administration), presumably due also to the insufficient elapse of time following the administration of CCl_4. In the present work we have not observed the relationship between the time after the intraperitoneal injection of hepatotoxic agent and changes in the liver weight or liver TG content, and no appropriate reference in this regard could be cited at this time. However, Stern *et al*[11] observed in rats that, when administered by stomach tube, CCl_4 caused progressive elevation of hepatic TG to reach the peak at around 48 hours; and Schotz *et al*[12] stated that within 1 hour after intragastric administration of CCl_4, liver TG increased 34%, and 195% within 3 hours, each as compared with contol in rat. In fact, the significant ($P<0.01$) loss of liver glycogen by CCl_4 under fed state revealed from the data of A' to those of C' group in our experiment certainly indicates the presence of hepatotoxic activity in the liver 6 hours post-CCl_4[4]. It should be emphasized that time factor following the administration of CCl_4 was not a single determinant that affected various liver parameters in this work but nutritional status prior to or after the injection of hepatotoxic agent should also be taken into consideration in understanding our data obtained from Expts I and II.

Conceivably fasting not only deprives external supply of energy but also consequently causes other vast metabolic changes in living organism. Although discussion on the whole effect of starvation on mammals is beyond the scope of present paper, it seems pertinent to state for the present discussion that fasting invariably results in reduction of blood glucose and concomitant

elevation of plasma free fatty acid (FFA) level.[13] It has become clear over the past decade that the FFA of plasma represent major form for mobilization of lipids from adipose tissue [14,15] and fatty liver can be produced by only raising the plasma FFA. [16] Elevation of plasma FFA in dogs induced by infusion of norepinephrine into the femoral vein was associated with a progressive and rapid rise in liver TG.[17] Studies of the fatty acid composition of the liver TG, plasma FFA, and adipose tissue TG were consistent with the finding that liver TG were synthesized from plasma FFA mobilized from adipose tissue[17]. Nestel *et al*[18] produced TG accumulation in the isolated rat liver *in vitro* by increasing the concentration of fatty acids in the perfusion fluid. Since lipogenesis was markedly attenuated in the liver of fasted rat[19,20] the source of increased unit weight liver TG observed in the fasted B and B' groups of present experiments should undoubtedly be derived from elevated plasma FFA in the fasting state, as discussed by Fritz.[21] Interesting enough, the 6-hour fasted A' group had higher liver TG content than that of the non-fasted A group, and 24-hour fasted B group had essentially the same degree of high liver TG content as 30-hour fasted B' group. From these it could be said that during the first 6 hours of fasting the liver TG had already started rising and by 24 to 30 hours it had reached plateau. On the other hand, the liver weight decreased progressively as fasting period prolonged; thus at 24 hours the decrease was approximately 10% (B vs A), and at 30 hours it was approximately 12% (B' vs A'). Our data contrast with those of Williams *et al*,[22] who showed that the liver weight loss in 24-hour fast in rat was 34% of original weight and 43 % after 48 hours.

The changes in the hepatic TG contents, as viewed from the point of fasting, in D (vs C) and D' (vs C') groups, were complicated by intervening CCl_4 administrition, as partly discussed previously. It was evident from our results as a whole, however, that whether the fasting was placed after (Expt I) or prior to (Expt II) the administration of CCl_4 its effect was aggravation of hepatic TG accumulation caused, through whatever mechanism, by the hepatotoxic agent.

The etiology for the accumulation of hepatic fats by CCl_4 has not been settled.[23] Chemical investigation revealed that rise in liver lipids following CCl_4 was due primarily to increased hepatic TG[12]. An indirect action of CCl_4, causing a sympathetic discharge with resultant increase in mobilization of fatty acids form the depot into the liver, as in fasting state discussed previously, had been suggested for the TG accumulation in the liver.[24~26] In fact, an increased incorporation of plasma FFA into liverTG was the major factor in the liver fat accumulation after surgical partial hepatectomy[27]. In CCl_4 poisoning, however, since plasma FFA concentrations were unaffected by CCl_4 treatment in the initial stage, or in view of time relationship between the onset of FFA increase and that of hepatic TG accumulation in the early initial stage by CCl_4, the "FFA theory" seems to be untenable[23,28]. Rather the body of evidence which has been reported in recent years strongly support the concept that CCl_4 causes fatty liver by directly acting on the liver to inhibit the hepatic secretory mechanism [28-36]. The hepatic TG secretion rate into plasma in CCl_4-treated rats was only 10% of normal[34]. In addition, soon after the ingestion of CCl_4 by rats it was observed that plasma TG level declined[29,36,37], presumably due to a block in the normal transfer of TG from the liver to the plasma; the striking elevation of plasma TG noted after the injection of Triton (which blocks the removal of TG from the circulation[38,39]) in control animal was absent.[29]

The sole source of fatty acids which accumulate as TG in the CCl_4-poisoned liver was

shown to be albumin-bound FFA of plasma, since no net gain of liver fatty acids resulted from the uptake of esterified fatty acids (TG)[40]. In addition, radioactivity administered as palmitate-1-C^{14} into CCl_4-poisoned rats was found in the liver TG fraction in high yield.[30,34,41,42] In CCl_4 poisoned liver, the enzyme system involved in activation of fatty acids to their fatty acyl CoA derivatives, transfer to α-glycerophosphate to form phosphatidic acid, dephosphorylation of the phosphatidic acid to form the α, β-diglyceride, and finally formation of TG by addition of the third fatty acyl CoA[43,44] seems intact.

Of the hepatic TG synthesis, apart from CCl_4 intoxication, the accumulation of TG by isolated perfused rat liver from a normal animal was directly proportional to the concentration of fatty acids in the medium over an wide range [45,46]. Then the synergistic or additive effect of CCl_4 and fasting in deposition of TG in the liver as observed in our results could be easily understood in terms of mass law action of elevated FFA brought about by fasting state. The increase in liver weight by CCl_4 in our experiments (C vs A, and D vs B; and C' vs A', and D' vs B') was opposed by the weight-decreasing effect of fasting (B vs A and B' vs A'), and resulted in compromised weight of slight increase in D and slight decrease in D', compared with that of C and C', respectively, which represent the action of CCl_4 only.

It could be said, therefore, that the elevated plasma FFA associated with fasting play role in the hepatic TG accumulation of the CCl_4-poisoned liver, although plasma FFA may not be the direct cause of fatty liver in CCl_4 intoxication as described previously. Our views are in accord with those of Weinstein *et al* [47] who reported lately that FFA concentration sufficient to induce accumulation of moderate amounts of TG in normal rat liver *in vitro* also caused retention of greater amounts of TG in the liver poisoned with CCl_4.

Summary

Lipid contents of carbon tetrachloride-induced fatty liver in mice were studied by placing the animals under 24-hour fast immediately after or prior to the intraperitoneal administration of the hepatotoxic agent or control substance. The following major points became evident from our studies.

1. previous or consecutive 24-hour fast with intraperitoneal injection of carbon tetrachloride induced accumulation of greater amounts of liver triglycerides than the simple administration of the hepatotoxic agent or 24-hour fasting alone would.

2. On the basis of our findings above, the requirement of free fatty acids for the development of fatty liver in carbon tetrachloride poisoning, apart from being a direct cause, seems inevitable.

In addition to these major conclusions, other gleanings obtained from our works were as follows.

1. The loss of liver weight by simple fasting in mice for 24 hours was by *ca* 10% and for 30 hours by *ca* 12%, of each control.

2. The gain in unit weight liver triglyceride content had already shown up by 6 hours after the induction of fasting and reached apparent plateau by 24–30 hours in relation to time course observed in two separate experiments.

3. The administration of carbon tetrachloride coupled with previous or ensuing starvation raised the unit weight liver total cholesterol content to a statistically significant extent; and fasting seemed to be dominating in effect over the action of carbon tetrachloride.

4. The unit weight liver phospholipid content was generally inversely proportional to the total liver weight except in the animal group admi-

nistered carbon tetrachloride with subsequent 24-hour fasting, in which group the phospholipid increase had in part contributed to the liver weight gain.

5. The unit weight liver glycogen content was most markedly decreased by the combined action of carbon tetrachloride administration and previous or subsequent fasting than any other experimental conditions set forth; however, the glycogen depleting action of carbon tetrachloride seemed to be superior to that of 24-hour fasting.

國 文 抄 錄

24 時間饑餓가 4鹽化炭素(CCl_4)中毒마우스肝臟의 各種脂質含量에 미치는 影響에 關한 硏究

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實驗動物에 CCl_4를 주면 肝組織의 損傷, 特히 急性投與時에 脂肪蓄積이 誘發된다는 것은 周知의 事實이고, 이러한 模型은 오래前부터 人體肝障碍機轉을 糾明하는데, 또 近來에는 脂質代謝를 硏究하는데, 이 方面의 硏究者에게 자주 利用되어왔다. 元來 營養狀態와 肝障碍間에는 密接한 關係가 있는 것이지만 CCl_4投與直後 또는 直前의 饑餓狀態가 CCl_4誘發脂肪肝에 量的으로 얼마나 關與할것인지를 밝혀내는것은, 서로 다른 實驗條件下에서 CCl_4中毒脂肪肝을 다룬 數많은 硏究論文의 結果를 個別的으로 解釋 · 理解하는데 있어서나 또는 相互比較하는데 있어서 重要한 問題가 될것이다. 또 饑餓가 CCl_4中毒脂肪肝에 미치는 影響을 量的으로 追求함으로써 CCl_4中毒脂肪肝發生의 病因論的機轉을 밝히는데 一助가 될 수도 있을 것이다.

實驗 I에서 마우스를 4群으로 나누어 A群은 對照, B群은 24時間單純饑餓, C群은 CCl_4單獨腹腔內注射, D群은 CCl_4腹腔內注射 및 그後 24時間饑餓로 處理하였다. 實驗 II에서도 實驗 I에서처럼 動物을 4群으로 分類하되 該當 24時間饑餓는 CCl_4腹腔內注射前에 挿入하고 CCl_4投與後 6時間에 該當되는 時期에 일제히 도살하였다.

實驗結果 얻은 知見은 要約 다음과 같다.

1. CCl_4投與直前 또는 直後의 24時間饑餓는, CCl_4單獨投與 또는 單純饑餓狀態보다 肝內 中性脂肪蓄積量을 훨씬 더 높였다.

2. 위 知見으로 보아 血漿游離脂酸은 CCl_4中毒脂肪肝發生에 이미 잘 알려 진 바와 같이 直接 原因的役割을하고 있진 않지만, 肝內 中性脂肪生合成過程에 材料가 되어 參與하는 것으로 생각된다.

3. 24時間 및 30時間饑餓로 마우스肝臟重量은 各各對照의 10% 및 12%가 減少하였다.

4. 肝臟單位重量當中性脂肪含量은 饑餓誘發後 6時間에 이미 늘기始作하고 24~30時間에는 上昇된 값에서 變動이 없었다.

5. 肝臟單位重量當總콜레스테롤含量은 CCl_4投與와 饑餓를 合併시켰을때만 有意하게 上昇하였고 兩要因을 分離하여 比較하면 CCl_4投與보다 饑餓로 더 上昇하였다.

6. 肝臟單位重量當燐脂質含量은 大體로 肝臟重量과 反比例的으로 變하였지만 CCl_4投與直後 饑餓를 合併시켰을 때만은 燐脂質含量增加가 肝臟重量增加와 더부러 있었다.

7. 肝臟單位重量當糖原質含量도 CCl_4投與와 饑餓를 併合시켰을때 가장 顯著하게 減少하였고 두 要因을 分離 · 比較하면 饑餓狀態보다 CCl_4投與로 더 減少하였다.

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