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EXPERIMENTAL STUDIES ON THE EFFECT OF FAT METABOLISM DUE TO LIVER INJURY

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In the organic metabolism, fat metabolism constitutes one of the important biologic process along with protein and carbohydrate metabolisms, hence, any abnormal condition which may arise in fat metabolism would likely to cause various diseases of metabolism. Fat metabolism of living organism has been known to comprises of various processes, such as digestion, absorption, synthesis, deposition, mobilization, oxidation and excretion; the fat substances when absorbed will enter circulatory blood through the portal vein or lymph vessel. The circulating fatty substance usually undergo various changes in the liver directly and are in a form of droplet which has 1 μ diameter to be distributed to the tissues.

In the fat metabolism, the role of liver is the most important as it is concerned with synthesis or decomposition of fat substances including cholesterol and in the production of bile, this speaks for paramount importance of functional integrity of the liver and its deranged function will affect faulty lipoid metabolism.

The present experiments was undertaken to investigate ability of the liver to dispose fat substance introduced in experimental induction of lipemia, causing disturbed hepatic function of central and peripheral lobules and with reticuloendothelial blockade.

This investigation was also combined with an attempt to clarify pathological physiology of the lobular unit.

METHOD

Experimental Animals:

Mature male rabbits which have average weight of 2 Kg. 78 were used. These animals were fed with a fixed food for a designated time and made experimental condition identical.

Method:

These animals numbering 78 were grouped into four and the following tests were made. In order to avoid effect of food, the animals were discontinued feeding 24 hours prior to test.

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First Group:

Rabbits No. 1–15: Straight fat injected group; Fatgen, the product of Dainippon Seiyaku, 20 cc. was slowly injected into rabbits' ear vein.

Second Group:

Rabbits No. 16–33; A group injected of the fat following colloid silver: 1 percent colloid silver 10 cc/Kg. was administered into animal's vein and then Fatgen 20 cc. was slowly given per vein 3 hours afterward.

Third Group:

Rabbits No. 41–58: A group injected of fat following pyrol: pyrol 0.5 cc/Kg. was subcutaneously injected in the animal's back and then Fatgen 20 cc. was slowly administered per vein 24 hours later.

Fourth Group:

Rabbits No. 61–78: A group injected of fat following allylformic acid: allyl formic acid 0.07 cc/Kg. was diluted with 100 times with phosiological salt solution and intraperitoneally injected, and then Fatgen 20 cc. was given per vein 24 hours after.

Before Fatgen injection, blood lipoid and cholesterol was determined of each group, and these were repeated following the injection in the intervals of 30 minutes, one hour, 2 hours, 3 hours and 24 hours, pre-injection determination was made with average serum of each group, post-injection estimation was done with average serum of the animals of same time intervals. Cholesterol determination was made with after Schörheimer et Sperry, while for lipoid, weight estimation method applied following ether expraction. (Ref: Table 1). Blood lipoid determination was done by taking blood specimens of respective group. These animals were sacrificed by means of air embolism. Necropsy was performed and the tissue specimens were removed and fixed in 10 percent formalin, and stained with hematoxylin (H. E.) and Sudan III for fats (S. III), and Smith-Dietrich (S. D.) for lipoids.

RESULT

I. *Quantitative Variation of Blood Lipoid and Cholesterol.*

As shown in the Table 1, determinations were made of lipoid and cholesterol contents in blood at pre and post administration times.

Blood lipoid content had increased steadily and reached twice the normal value by two hours when fat alone was given and even after 24 hours, the value remained high. Cholesterol, on the other hand, showed slower elevation for the first hour but after two hours, it rapidly rose and increased as much as double the normal value by 3 hours and it continued to stay high even after 24 hours.

With colloid silver administration and accompanying blocking of the reticuloendothelial system, the administration of fat caused increased lipoid content beginning from 30 minutes to one hour and it would reach twice the former value

and continues to remain. Cholesterol, however, was less variable up to two hours, it would show a highest value after 3 hours and decrease afterward resuming the original value by 24 hours.

Table I

Experimental animals No.	Experimental method	Survival Time	Normal		Before Fat Adm.		After Fat Adm.	
			Ch. $\frac{mg}{dl}$	Li. $\frac{mg}{dl}$	Ch. $\frac{mg}{dl}$	Li. $\frac{mg}{dl}$	Ch. $\frac{mg}{dl}$	Li. $\frac{mg}{dl}$
13 14 15	A group injected	½ h	67	220			67	280
7 8 9	fat only	1 h					67	360
4 5 6		2 h					84	550
1 2 3		3 h					151	360
10 12 12		24 h					133	680
31 32 33	A group injected	control			59	100		
25 26 27	of fat following	½ h					67	120
19 20 21	colloid silver	1 h					67	280
22 23 24		2 h					67	270
16 17 18		3 h					100	350
28 29 30		24 h					67	370
56 57 58	A group injected	control			84	160		
53 54 55	of fat following	½ h					107	140
50 52	pyr l	1 h					122	140
44 45 46		2 h					71	120
41 42 43		3 h					80	
49 51		24 h					80	82
	A group in ected	control			59	120		
70 71 72	of fat following	½ h					67	40
64 65 66	allylformic acid	1 h					67	160
67 68 69		2 h					94	30
61 62 63		3 h					80	170
73 74 75 76 77 78		24 h					157	142

With pyrol pretreatment causing liver injury 24 hours before fat administration revealed less lipoid change and little decrease noted even after 24 hours. This was not the case with cholesterol, it indicates a highe value in less than 30 minutes or one hour. It showed marked rise two to three hours and remained the similar value.

With pretreated allyl farmic acid group, lipoid increase was usually seen shortly after 30 minutes to one hour but reached roughly three times concentration which would remain after 24 hours. Cholesterol showed rise in one-two hours, remaining approximately the same value up to 3 hours; it continued to show increase after 24 hours reaching roughly double the initial value.

II. *Histological Findings.*

A. Fat Stained Rabbits for Control.

Except for occasional stainable fat to be seen in the Kupffer's stellate cells, and practically no fat to be observed in normal stellate or hepatic cells with S. ⁝. stain for fat.

In the lung tissues, no stainable fat could be observed in the epithelium of alveolar or bronchial tissues with both Sudan III or S. D. stains. With the former, however, a trace of fat was visible in the peribronchial cartilagenous tissue.

In the spleen, stainable fat was found to be entirely negative with either stain in white or red pulps.

B. Straight Fat Injected Group.

1. Fat Stain.

Liver.

Thirty minutes after fat injection free fat droplets could be seen in the sinusoid but after one hour, these were no longer to be find. Stainable fat began to appear mainly in lobular periphery and then central area one hour later but became most marked 2–3 hours later. Gradual reduction was seen 24 hours later and only a trace would be remained. The stellate cells were high in fat content and show much swelling, cytoplasm contained of numerous fine fat granules. The nuclei appear to be compressed. The hepatic cells failed to show fat content in general, if there is any, it was seen in the lobular periphery two or three hours later but of extremely small in some areas.

In the Glisson's cupsules, fat histiocytes were seen occasionally, otherwise, no stainable fat to be seen, the stellate cells found in the peripheral lobules were shown to be positive with fat with S. D. stain. The hepatic cells in these particular areas also showed slight positive fat one to two hours.

Lung.

In the blood capillary of alveolar septum, a small amount of free fat droplets could be seen 30 minutes after fat administration. Increase was more noticeable 1–3 hours later in the alveolar epithelium with S. III stain. Twenty four hours later, however, hardly any fat granules could be seen. Phagocytic content of fat in alveolar epithelium was much higher than those found in Kuffer's cells. Only one case showed fat in bronchial epithelium after two hours, and none in rest of them. S. D. stainable fat in alveole was usually seen reaches most marked after 2–3 hours and retains little after 24 hours except in some cases which showed only slight. In all cases, the bronchial epithelium had failed to show.

Spleen.

Free fat droplets were detectable in the blood vessels 30 minutes after the administration but none after that. In the reticular cells, it began to show up by 30 minutes and markedly increased but little to be seen after 24 hours. It is more congregated in the periphery of pulp, particularly in the reticular cells,

free splenocytes and the lining cells of sinus in the red pulp. In some cases, very little droplets were seen in the pulps after two hours. S. D. stain demonstrated fat in the reticular cells of the cords one – three hours but none to be observed 24 hours. It was not seen in the tissue of white pulp.

2. Hematoxylin Eosin Stain

Liver.

Alightment of the hepatic cells were regular and no sign of cloudy swelling to be seen. Fine vacuoles have been seen in the central or intermediate zone of lobules. Some cells presented foamy appearance. No necrosis could be observed. Many of the stellate cells became swollen and contained vacuoles having nuclei compressed to one side in semilunar fashion. No noticable changes to be observed of the nuclei of hepatic cells specially around lobular periphery. In the si-

	hour	No.	liver										lung				spleen			
			central parts of the hepatic lobules					periphery of the hepatic lobules					respera-tory epithe-lium		bron-chial epithe-lium		splenic pulp		splenic cor-puscle	
			stellate cells		liver cells			stellate cells		liver cells										
			S. Ⅲ	S. D.	S. Ⅲ	S. D.	necrosis	S. Ⅲ	S. D.	S. Ⅲ	S. D.	necrosis	S. Ⅲ	S. D.	S. Ⅲ	S. D.		S. D.		S. D.
a group injected fat only	½	13	−	−	−	−	−	−	−	−	−	−	+	++	−	−	+	+	−	−
	½	14	−	−	−	−	−	−	−	−	−	−	+	±	−	−	+	±	−	−
	½	15	−	−	−	−	−	−	−	±	−	−	−	±	−	−	+++	±	−	−
	1	7	±	−	±	−	−	+	±	±	±	−	++	+++	−	−	++	+	−	−
	1	8	±	−	±	−	−	+	±	±	−	−	+++	+++	−	−	+++	++	±	±
	1	9	±	−	¬	−	−	+	+	+	±	−	++	++	−		+	++	−	−
	2	4	+	+	±+	−	−	++	+	±+	+	−	++	++	−	−	++	++	±+	+
	2	5	±	+	±	−	−	+	+	±	±	−	++	++	++	−	+++	++	±+	−
	2	6	+	++	+	−	−	+	++	+	±	−	++	+++	−	−	+++	++	±	−
	3	1	−	+	−	−	−	++	+	−	+	−	+++	+++	−	−	++	++	−	−
	3	?	+	−	±	−	−	+	±	±	±	±	+++	||||	−	−	+++	+	−	−
	3	3	+	−	±	−	−	+	±	±	+	−	++	+	−	−	+++	+	−	−
	24	10	−	±	±	−	−	+	−	±	−	−	±	+	−	−	+	−	−	−
	24	11	±	−	±	−	±	−	±	±	−	−	−	±	−	−	±	±	−	−
	24	12	±	−	−±	−	−	±+	−	−±	−	−	+	+++	−	−	/	−	/	

D. Ⅲ---Sudan Ⅲ staining for neutral fats

S.D. Smith-Dietrich's method for lipoids

nusoids, were many free polymorphnuclear leucocytes wandering. The Glisson's capsules showed vascular filling but no sign of proliferation of connective tissue or abnormal finding of bile ducts or cellular infiltration.

C. Group Injected of Fat Following Silver.

1. Fat Stain.

Liver.

Fat content appears in the Kuffer's stellate cells by thirty minutes, no evidence of free sinusoidal fat to be seen. Those stellate cells of high fat were mostly found in the lobular periphery, but lesser in central area. Increase was prominent after 30 minutes, kept fairly steadily up to three hours but lost by 24 hours. With S. D. stain it is detected in the lobular periphery by one hour and also central lobule. Following introduction of fat it reached the highest by 2-3 hours and only a trace could be seen by 24 hours. Sudan III stain showed mainly in the periphery but very little in amount, though distributed well and none was coarse type. With S. D. stain only small amount was seen in the peripheral lobule in some cases after 2-3 hours. Fat engulfed histiocytes were seen in the Glisson's capsules, but none to be seen in the epithelium of the bile ducts.

Lung.

Although alveolar capillaries contained no free fat but it was definitely positive in the alveolar epithelium with S. III stain. It was most abundant in during 2-3 hours and lasts for three hours and only very small amount could be detected after 24 hours which were limited in some areas and most were negative. With S. D. stain only the limited alveolar epithelium could show mostly became marked in one to three hours there were much clumps of fat appeared in this group. Both stains showed negative fat in bronchial epithelium.

spleen.

With S. III stain, fat could be detected in the reticular cells after 30 minutes but none found in the vessels. It is more prominent in the tissue around the white pulp and not in pulps themselves. The cells of cords containing fat were reticular cells, free splenocytes and lining cells of sinus, most pronounced in the group after 30 minutes or 3 hours. After 24 hours only the splenic cord held fatty droplets.

S. D. stainable fat was mostly seen in the cord after one hour, became less during 2-3 hours continued to stay, only a small amount remained in cord cells, and not a single case remained positive in the cells of pulp with S. D. stain.

2. Hematoxylin Eosin Stain.

Liver.

Liver cells arranged regular and cloudy swelling was shown only in one case, which were swollen in the lobular periphery and cellular boundary was indistinguishable. In a small number of cases vacuoles were formed in the periphery of lobules. With this group, however, small round cell infiltration in the lobular periphery had been observed, Mild nuclear pyknosis was seen in certain number

of cases. The stellate cells were mostly swollen with small vacuoles. In some of them contained fine dark brownish granules within the cells, other were lacking, Wandering of leucocytes were frequently seen in the sinusoids. In small number of Glisson's capsules presented the picture of small round cell infiltration but remainders including bile ducts showed no abnormal condition.

	hour	No.	liver										lung				spleen			
			central parts of the hepatic lobules					periphery of the hepatic lobules					respiratory epithelium		bronchial epithelium		splenic pulp		splenic corpuscle	
			stellate cells		liver cells			stellate cells		liver cells										
			S. III	S. D.	S. III	S. D.	necrosis	S. III	S. D.	S. III	S. D.	necrosis	S. III	S. D.	S. III	S. D.		S. D.		S. D.
a group injected of fat following colloid silver	½	25	±	−	±	−	−	+	±	±		−	##	+	−	−	##	+	−	−
	½	26	+	+	−	−	−	##	+	−	−	−	##	+	−	−	+##	+	−	−
	½	27	−	±	+	−	−	±	±	+	±−	−	±+	±	−	−	####	±	−	−
	1	19	+	+	±	±	−	+	+	±	±	−	##	####	−	−	+	##	−	−
	1	20	±	±	±	−	−	+	±	±	−	−	+	##	−	−	+##	±	−	−
	1	21	±	±	±	−	−	+	+	±	+	−	+	####	−	−	+	##	−	−
	2	22	±	±+	±	−	−	+	±+	±	−	−	###	####	−	−	+	±	−	−
	2	23	+	+	±	±	−	+	+	±	+	−	##	####	−	−	+	±+	−	−
	2	24	−	+	±	±	−	±	+	±	+	−	+	+	−	/	/	±+	/	−
	3	16	±	+±	±	−	−	+	+±	±	±	−	+	##	−	−	##	+	−	−
	3	17	±+	±	±	±−	−	+	+	±	+	−	###	####	−	−	+##	±	−	−
	3	18	±	+	±	−	±	+##	+	±	##	−	######	####	−	−	+	+	−	−
	24	28	±	+±	−	−	−	±+	+±	−	−	−	+	##	−	−	+	+	−	−
	24	29	+	±	±	−	−	+	±	±	±	−	+	####	−	−	+	##	−	−
	24	30	±	−	+	−	−	±	−	+	−	−	−±	±	−	−	±+	±	−	−

S. III—Sudan III staining for mutual fats
S.D. Smith-Dietrich's method for lipoids

D. Group Injected of Fat Following Pyrol.

1. Fat Stain.

Liver.

With Sudan III stain free lobular fat globules failed to show after 30 minutes because of a fact that up to this time these were mostly taken up by the Kupffer's cells and which is less than the amount to be absorbed by the pulmonary alveolus. Fat contained stellate cells appear to be more distributed in the lobu-

lar periphery. The amount is increased one-two hours and reaches the highest aronnd 3 hours. It is still detectable even after 24 hours though limited. In the liver cells, 4 out of 14 cases have undergone necrosis of central lobules which contained coarse granular fat droplets. Even with other fat content is more noticeable but never to a degree of forming droplets. Occasionally, histiocytes which have englufed unusually small fatty granules were seen in the connective tissue of Glisson's capsule but none found in bile duct epithelial cells. With S. D. stain fat found in the stellate cells show bluish tint after one hour which was most visible in the periphery. In the liver cells, however, it was faintly positive in the lobular periphery after one – two hours.

	hour	No.	liver										lung				spleen			
			central parts of the hepatic lobules					periphery of the hepatic lobules					respiratory epithelium		bronchial epithelium		splenic pulp		splenic corpuscle	
			stellate cells		liver cells			stellate cells		liver cells										
			S. III	S. D.	S. III	S. D.	necrosis	S. III	S. D.	S. III	S. D.	necrosis	S. III	S. D.	S. III	S. D.		S. D.		S. D.
a group injected of fat following pyrol	½	53	–	–	±	–	+	±	±	±	–	–	±+	++	–	–	±	±~	–	–
	½	54	–	–	±	–	+	±	±	±	–	–	+	++	–	–	+	±	–	–
	½	55	±	–	++	+	++	±+~	+	++	–	±	+	++	–	–	+	++	–	–
	1	56	±	±	±	–	+	+	+	±	–	–	++	+++	–	–	+	+	–	–
	1	57	±	±	±	–	+++	+	+	±	–	±	±	+	–	–	+	+	–	–
	1	58	±	±	±	–	±	+	–	±	–	–	+	++	–	–	+	+	–	–
	2	44	–	+	±	–	+	+	+	±	–	–	++	++	–	–	+	+	–	–
	2	45	±	±+~	±	–	±	+	±+~	±	–	±	+	+++	–	–	+	++	–	–
	2	46	–	+	+	–	+	+	+	±	–	–	+	++	–	–	+	+	–	–
	3	41	±+~	±	±	–	±	±+~	++	±	–	–	+	+++	–	–	+	++	–	–
	3	42	±	+	±	–	±	+++~	+	±	–	–	+++~	+++	–	–	+	++	–	–
	3	43	+	±	±	–	+	++	+	±	–	–	+	++	–	–	+	+	–	–
	24	49	–	–	++	–	+++	±	±	–	–	±	+	++	–	–	±~	±	–	–
	24	51	±	±	++	–	±	+	±	±	–	–	±	+	–	–	±	±	–	–

S. III—Sudan III staining for neutral fats
S.D.—Smith-Dietrichis method for lipoids

Lung.

The fat was mainly taken up by the alveolar epithelium and no free fat could be detected in blood vessels. The increase was noticeable after one hour and up

to three hours; after 24 hours only small amount in certain parts showed fat droplets. No fat was found in bronchial epithelial cells.

With S. D. stain, the fat became positive after 30 minutes, increasing steadily up to 3 hours, the highest, and by 24 hours it was still retained considerably. It was entirely negative in the bronchial epithelial cells.

Spleen.

S. III stain showed that most of fat grobules have been taken up by reticular cells of red pulps, splenocytes and endothelial cells of sinus after 30 minutes. No free globules could be seen in the sinus. These grobules increase up to one –two hours, and by 3 hours, it began to reduce and none present after 24 hours. These droplets were detectable after one hour and reaches strongly positive by 3 hours with S. D. stain. It is no longer visible in the reticular cells after 24 hours. In the pulps neither S. III nor S. D. stain proved any evidence of fat in all instances.

2. Hematoxylin Eosin Stain.

Liver cell arrangement is regular on the whole except in those cells cords where necrosis present. One third of the group showed hepatic necrosis which was mostly found in central lobular zone or intermediate part, in some cases, necrosis extends to the periphery. These necrotic cells were disintegrated showing granular or vacuolar broken down elements with indistinguishable boundary. The nuclei were swollen with vacuoles undergone pyknosis. Congestion and mild bleeding accompanied in the necrotic lesion. The stellate cells in these areas retained protoplasmic structure fairly well but they were swollen with vacuoles and many rook semilunary appearance of nucleus.

E. Group Injected of Fat After Administration of Allyl formic Acid.

1. Fat Stain.

Liver.

With S. III stain, fat taking pale orange color could be detectable in some parts of sinusoids and lobular venules of the Glisson's capsules after 30 minutes but no longer visible after one hour. In the stellate cells found in the lobular periphery fat was visible by 30 minutes which was most pronounced after 2 – 3 hours. Only in the limited cases, it was markedly positive in those found in central lobules.

After 24 hours, however, only one case demonstrated fat granules and none other showed any sign of fat. Roughly half of hepatic cells have undergone necrosis in the lobular periphery. These have presented with coarse for granules. Fat containing histiocytes were seen in the Glisson's capsules sparingly. In some parts of bile duct epithelium had contained fat also.

With S. D. stain, fat droplets were positive in the stellate cells after one hour, bluish color got deeper up to 3 hours and after that it was lost. In the liver cells, however, such diffuse bluish color was not seen but the stellate cells be-

came most visible after 2–3 hours. In some cases blue color extends into liver cells seen around the stellate cells. It is not stainable in necrotic liver cells or Glisson's capsules.

Lung.

With S. Ⅲ stain fat was weakly positive in some parts of moderately sized veins of pulmonary circulation by 30 minutes to hour after fat injection. This is no longer positive after 2 hours. Fat globules were seen in abundance in alveolar epithelium, these reach highest 2–3 hours and continue remain with little decrease. Bronchial epithelium failed to show fat granules.

With S. D. stain for contained in pulmonary epithelium turned pale blue after 30 minutes, reaching the highest as the time elapsed and continue to remain so

	hour	No.	liver										lung				spleen			
			central parts of the hepatic lobules					periphery of the hepatic lobules					respiratory epithelium		bronchial epithelium		splenic pulp		splenic corpuscle	
			stellate cells		liver cells			stellate cells		liver cells										
			S. Ⅲ	S. D.	S. Ⅲ	S. D.	necrosis	S. Ⅲ	S. D.	S. Ⅲ	S. D.	necrosis	S. Ⅲ	S. D.	S. Ⅲ	S. D.		S. D.		S. D.
a group injected of fat following allyl formic acid	½	61	±	−	++	−	−	±	−	++	−	++	+	+	−	−	±+	±	−	−
	½	62	+	±	±	−	−	+	−	±	−	−	+	++	−	−	+	+	−	−
	½	63	±	+	±	−	−	±+	±	±	−	±	+	++	−	−	±+	+	−	−
	1	64	±	+	±	−	−	++	+	±	−	+	+	+	−	−	+	±	−	−
	1	65	±	−	±	−	−	+	+	±	−	−	+	+	−	−	+	±	−	−
	1	66	−	−	−	−	±	±	+	++	−	±	+++	+	−	−	+	±+	−	−
	2	67	+	−	±	−	−	++	−	±	−	++	++	±	−	−	++	−	−	−
	2	68	±	+	±	−	−	++	+	±	−	+	+++	+	−	−	+	+	−	−
	2	69	±	+	±	−	−	++	+	±	−	−	++	++	−	−	+	+	−	−
	3	70	±	+	−	−	−	++	+	±	−	−	+	+	−	−	+	+	−	−
	3	71	−	−	±	−	−	+	−	±	−	+	+	+	−	−	++	−	−	−
	3	72	±	++	±	−	−	+++	++	±	−	−	+++	++	−	−	+	++	−	−
	24	73	−	±	−	−	±	+	±	++	−	++	+	++	−	−	+	±	−	−
	24	74	±	−	±	−	−	±	−	±	−	±	+++	++	−	−	±	±	−	−
	24	75	+	−	±(+)	−	−	++	−	±(+)	−	−	++	++	−	−	++	±	−	−
	24	76	±	+	−	−	+	±	±	++++	−	++	+	++	−	−	+	±+	−	±

S. Ⅲ – Sudan Ⅲ staining fcr neutral fats
S. D—. Smith-Dietrichis method for lipoids

even after 24 hours. Bronchial epithelium was found to be negative.

Spleen.

With S. III stain, free fat was detectable in a limited area of sinus but it soon lost and no longer detectable by one hour. Practically all of fat introduced have been taken up by the reticular cells. There was no quantitative fluctuation of fat absorbed by these cells of pulps throughout the period. After 2–3 hours, however, a few cases turned real positive. These cells still held much fat even after 24 hours.

With S. D. stain only mild for reaction of pale blue could be seen in the reticular cells. None of these cells were marked as to show throughout an entire period but these could be visible up to 3 hours. These cells failed to show real blue positive coloration by 24 hours. Fat was not seen in the pulps.

2. Haematoxylin Eosin Stain.

Liver.

While no special changes to be noted in the central and intermediate lobular arear only mild vacuolization had been observed. The peripheral liver cells have undergone necrotic changes in cord like manner trapping Glisson's capsuls; some have shown colliquative necrosis while others were pyknotic or lost. Some cells have shown either acidophilic coagulation or partial vacuole formation. Cellular reaction or hemorrhage were least seen. The Glisson's capsules in this stage showed little change and proliferation of the connective tissue was not seen.

In the necrotic areas were seen some swelling of Kupffer's cells and also found wandering polymorphnuclear leucocytes in the sinusoids.

DISCUSSION AND SUMMARY

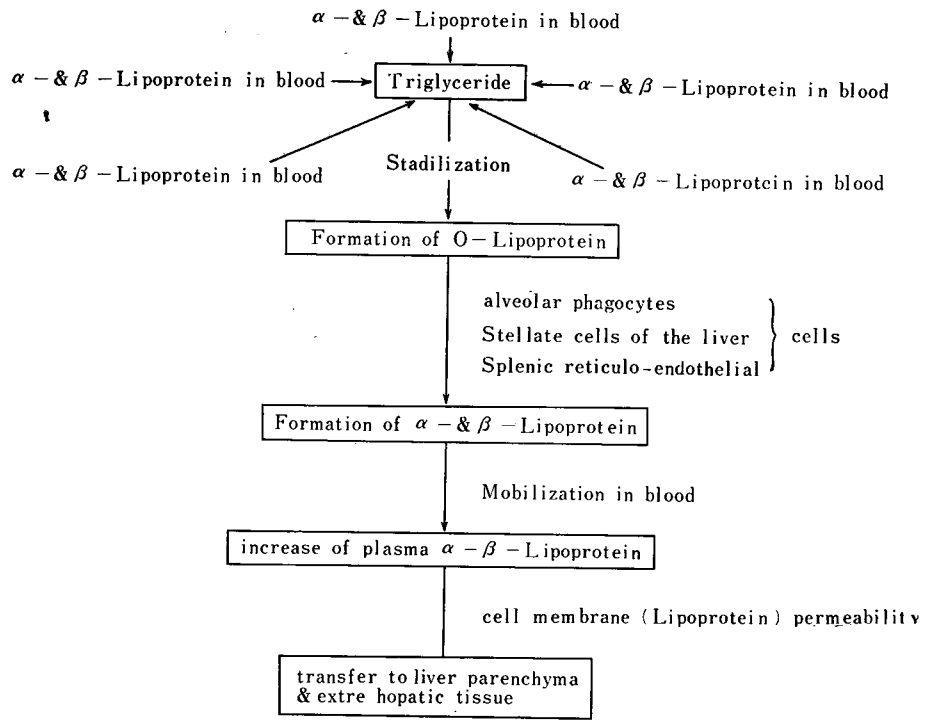
Fat substance with comparises a part of cellular protoplasm of living organism are made up of phospholipid, glycolipid, and cholesterin. These are essentially for structural or functional purposes. While, the fat which is to be used as metabolic purpose supplying source of energy are mainly consists of palmitic acid, palmitooreic acid, stearic acid and oreic acid, and their respective glycerine esters.

Recently, Higasa has made an extensive study on metabolism and manufacture of an artificial fat, an emulsified fat which is transportable to be introduced directly into vein of postperative patients who required of nutritive fluid non-parenterally.

Metabolic process of fat involves a chain of biochemical actions i. e., digestion, absorption, synthesis, deposit, mobilioation, oxidation and elimination. But when emusified fat substance was introduced directly into the vein, the processes involved would be different from the oral administration of fat of any type. It will be circulated the entire system in least possible time, there it will soon taken up by the stellate cells of Kupffer, reticular cells of the spleen, the reticuloendothelial, free splenocytes, sinus endothelial and pulmonary alveolar phagocytes. The mechanism of deposition of fat in those tissues can be explained that in the lung

capillaries blood pressere is lower than that of aorta or peripheral capillary pressure, which will likely to cause stasis of fat which in turn would facilitate to take up more fat due to dilatation of vascular wall with lesser resistance. In the liver and spleen, the vessels dilate, thus, blood velocity is reduced and the fat globules undergo cellular disposal.

The fat injected could be detectable in large amount after 30 minutes, then, began to decrease one hour later and continue to be so until 2 hours, by 3 hours it is practically gone and by 24 hours it was completely disappeared. Hence, it is possible to assume cellular fat disposal is usually completed around 3 hours; the same can be said of other organs such as lung, liver and spleen. Among these tissues, uptake of fat is most striking with lung tissue within a fixed time, then comes, spleen and liver, in other word, pulmonary tissue is possessed of a remarkably high ability to dispose fat. Histochemical method has revealed a fact that the cellular fat would be more S. D. stain positive along with elapse of time. The fact seemed to explain that those neutral fats found in the reticuloendothelial system, decompose into fatty acids which in turn would synthetize into lipoid substance. In this regard, Higasa explained disposal of fat from biochemical aspect by saying that the triglyceride when injected into vein, first, α - and ß - lipoprotein of serum accumulate and forming 0 - lipoprotein which is a stabilized from of fat taken up by alveolar phagocytes, Kupffer's stellate cells of the liver and spleen.



(adapted from Higasa)

These will be in circulation after glyceride had became shifted into phosphatides which is no longer a free form of phosphatise but exist as α - and β - liprotein and transported throughout the system.

It has been called to our attention that the lung might have some specific role and intimately related with fat metabolism, basing on the physiological fact that a major part of absorbable fat from the intestinal tract would enter the thoracic duct then to venous channel and pass through the lung. Roger et Binet have advanced an idea of socalled Function Lipopexique, Function Lipodié végique of the lung on the fact circulatory fat is decreased almost 15 percent during pulmonary circulation.

Abelous, Soula, Remondand Bernardberg have demonstrated that cholesterol content of the right heart is greater than of the left heart. While, Derman, Leiles, Jankovich, Jeckeln, Butterworth and Sungley have reported that the lung presumably engaged in decomposition and excretion of fat substance from the finding that the unusual amount of fat granules were included in the alveolar phagocytes in lipemia cases.

Higasa, in this correction, assemed that the lung may have functional role of adjusting blood cholesterol level from the evidence that when a large amount of emulsified fat was given intravenously numerous fat inclusion alveolar phagocytes were detached and cholesterol would be eliminated. On the contray, Markowitz, Mann and MacLachlan were those who have expressed oppossing view in this regard.

Although the writer has failed to substantiate either cholesterin being eliminated in the alveolar space or bronchioles but found a large amount of fat could be taken up by the pulmonary tissue and converted into S. D. stain positive phosphatide and that such function would be heightened to some extent when hepatic dysfunction is present. The finding does seem to support the view that the lung is cupable of disposing exogenous fat when in excess.

As to the role of spleen for fat disposition, its process is similar to that of uptake performed by reticuloendothelial tissue primarily. Kondo reported markedly lowered functional capacity of taking of fat by either reticuloendothelial blockade or splenectomy. Fligenboum, on the other hand, in order to study the ability of reticuloendothelial system to dispose dietory cholesterol in mammals, he had used indian ink to block the system and found delayed in fat disposal of blood cholesterol due to interference. Recently, Higasa had stated that when the endothelial blocking was relatively effective with ink, initial disposition of fat is most likely to be delayed and some degree of disturbance will be the case during oxidizing process. These have pointed out the fact that splenic ability of fat depend upon either increase or decrease of primary disposition of fat.

The function of liver is said to have associated with intermediate metabolic process of fat. No doubt, the liver should engage a significant performance if any fat substance is directly introduced by venous route. Higasa, in his investigation made known that in such instance, most of fat substance would be ingested

by the Kupffer's cells in the first place and only a part would be trapped directly by liver cells; and that the liver cells would eventually ingest it in a form of phosphatide which had undergone above initial treatment. It involves two stage processes, the one chiefly by the reticuloendothelial cells and the other by the liver cells themselves. Jaffe and Bergman have brought out the vital role of Kupffer's stellate cells in connection with fat metabolism and they have stressed the importance of functional until between the above cells with hepatic cells. Friedman stated that the stellate cells of Kupffer often undergo lipotrophic changes in deranged fat metabolism and maintained that the cells may act as significant part in accumulation of exogenous cholesterol. Recently, Ito reported of an existence of an independent lipotrophic cells which are said to be closely associated with lipoid metabolism. It has been known that the liver cells have functional adaptability in relation with their venous lobular structure. According to Yamada, in the condition lipemia due to starvation or dietary intake, lipoid infiltration usually begins in the lobular periphery, then extends to the central area but the process is reversed that is from the central to the periphery in the condition of lipoid consumption and its retention in the periphery of lobules is the longest, such an observation was verified by Nishimura and stated that the reason for lipoid fluctuation in the lobule as well as reference to lipase test finding are the signs of heightened hepatic function of this area and in all probability attributed to peculiar vascular distribution of the lobules which offer to accelerate fat intake of hepatic cells.

The author has awared that the stellate cells of lobular periphery were filled with lipoid droplets in the early stage and quantitatively abundant in comparison with central part. In this connection, Hara et Koga have made comparative studies on the metabolic capacity of the lobules with reference to functional variation between the central and periphery of the lobular areas by injuring respective area. They have stressed specific function of the liver lobular area the lobular function is severely injured when the central lobule has been disturbed with pyrol injection but with lesser secretory and excretion injuries, but when the lobular periphery is injured with aryl formic acid secretory and excretory functions are markedly affected but with lesser metabolic disturbances.

Much has been studies on the quantitative variation of blood cholesterol in liver disturbance. A. Flint, Thannhanser, Schaber, Adler, Stroebe and Spstein and the others have reported on low blood cholesterol content in liver injury. While, Chanffard, Feigi and Bang and the others have reported on the elevated value of cholesterol content in obstructive jaundice along with hyperbilirubinemia, an useful procedure in differential diagnosis. The high value could be attributed to accumulated cholesterol but it is not simple as that add more complicated mechanism seemed to be involved.

Taking into consideration of these facts and together with present results, the author was able to point out the following summary. That quantitative variation

of lipid could be classified into two types. That is, the one type of which blood lipid has rose steadily with the time element following administration of fat as observed in those cases straight fat or colloid cilver, and the other type in which blood cholesterol did not show marked fluctuation on the whole such as observed in pyrol or allyl formic acid. From the author's histochemical finding as well as Higasa's histological and histochemical studies, it is possible to assume that the lipid quantity estimable one hour after fat administration is no way related with administered fat but rather by the fat value obtained after disposal by reticuloendothelial cells. The reason for less increase of fat in the group with administered by colloid silver compared with straight fat injected group is perhaps due to the fact that the reticuloendothelial tissue may have been functionally imparied and reduced an ability of far disposal. On the other hand, when the liver has been damaged by pyrol or allyl formic acid administration and no lipid elevation to be seen may have been lesser disposing capacity of hepatic tissue due to disturbed function. In this instance, it made no difference whether liver injury was found in the central lobule or periphery.

While, cholesterol variation does not follow closely that of lipid, but its quantity roughly follows, though some variation was admitted in these cases in which fat was administered after blocking or reticuloendothelial system by straight fat or colloid silver. That is blood cholesterol content has arose 2 - 3 hours after fat administration and then, showed gradual decrease, whereas, if treated previously with pyrol or allyl formic acid, somewhat different variation would result. There seems to be an opposite relation between pyrol administered group and allyl formic acid group: the former reaches the maximum by 30 minutes to one hour and show reduction between two to 24 hours, on the contray, the latter show gradual increased between 2 - 3 hours and reaches the maximum by 24 hours.

This speaks for the fact that pyrol would chiefly injure the liver cells of central lobule while allyl formic acid wonld affect peripheral lobule. According to Hara, when the lobular periphery is injured, bile excretion would be affected accordingly which is unlike that of central lobule.

The author has also observed higher cholesterol value in cases of lobular periphery disturbance with pyrol as we find in cirrhotic liver, the condition may be of the same situation, however, further investigation is necessary for the cause.

In the histochemical studies of liver injury, loss of Sudan III stainable fat and S. D. stainable fat phagocytosed by the reticuloendothelial system were delayed as compared with straight fat injection cases, it is probably due to later reduced ability to dispose fat as the initial phagocytic ability of reticuloendothelial system has been shown. Of the various causes, one may be the reduction of lipase found in the liver cell may have the reason. With liver injury, tendency for pulmonary cell and reticular cells of spleen to accelerate phagocytic function is probably the result of compensatory hyperfunction of reticular cells to take up more blood fat.

CONCLUSION

1. Experimental studies of metabolic process of fat with special reference to the disposition of fat together with quantitative estimation of blood lipid and cholesterol by intravenous administration of Fatgen an emmulsified fat agent using rats were made and histochemical examination of organs were also made.

2. It was found that the emulsified fat when intravenously introduced would mainly taken up by reticuloendothelial cells of the lung, spleen and liver and usually lost from 30 minutes to one hour, and then, following the disposition by these cells, they undergo changes from Sudan III stainable glyceride to S. D. stainable phospholipid and liberated in circulatory blood again. A part of these would be taken up by hepatic cells directly.

3. Blood lipid usually elevates after 30 minutes; cholesterol rose suddenly after one - two hours and tend to decrease.

4. When the emulsified fat agent was injected after preliminary blockade of reticuloendothelial system with colloid silver, the function of fat disposal was somewhat reduced and the time for disappearance of blood lipid would be delayed. Although blood lipid increases after one hour but slightly lower than control value. Cholesterol value, however, increases for time being after three hours but resumes the former value after 24 hours.

5. The cells of reticuloendothelial are reduced of their ability to dispose fat and delay the time for disappearance when the liver was injured with pyrol and allyl formic acid and followed by injection of the fat emulsion. The blood lipid content is markedly lowered compared with the control group.

6. No particular quantitative variation of blood lipid observed when the central lobules of the liver was injured with pyrol. Cholesterol was increased strikingly after 30 minutes to one hour and decrease again after 2 hours.

7. Blood cholesterol showed slight increase after one hour when the lobular periphery of the liver was injured. Cholesterol began to show an increase after one hour and markedly increased after 24 hours.

9. With the liver injury whether the involvement is of central or periphery, an increase of blood lipid is extremely small contrasted with the controls. The blood cholesterol had increased exceedingly when the lobular periphery was injured with allyl formic acid as compared with the case injured of the central.

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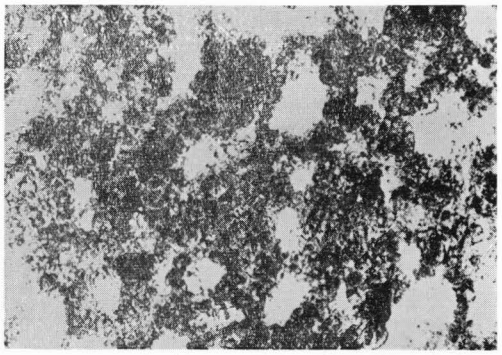

Fig. 1. Lung (No. 8) Many alveolar phagocytes, containing a large quantity of sudanophilic neutral fats, are seen.
Sudan III Staining.

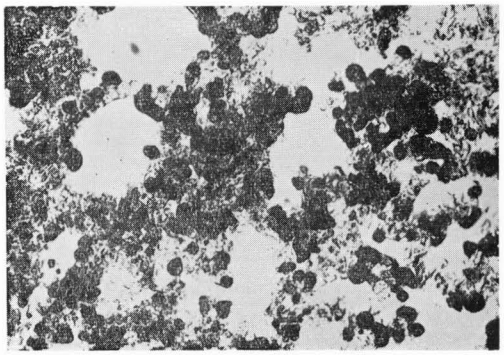

Fig. 2. Lung (No. 6) Sudanophilic neutral fats in alveolarphagocytes gradually change into Smith-Dietrich's method positive staining fats. S. D. Staining.

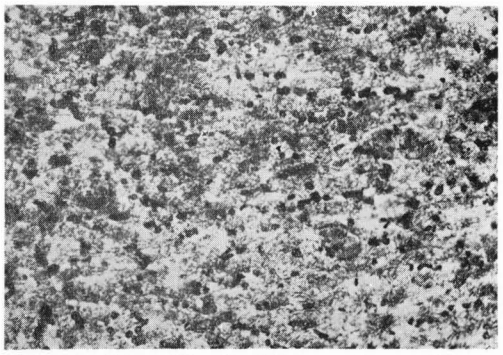

Fig. 3. Liver (No. 1) Many stellate cells of Kupffer, swollen by taking fatty droplets are seen.
Sudan III staining for neutral fats.

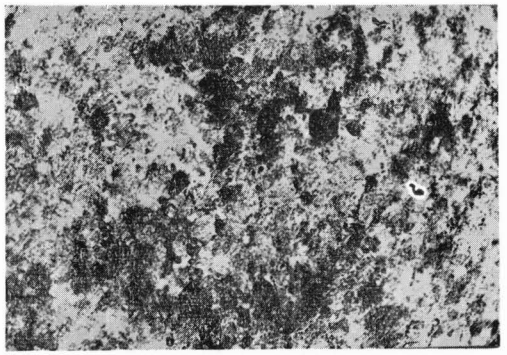

Fig. 4. Liver (No. 6) Fats in the stellate cells of Kupffer gradually c ange into Smith-Dietrich's positive staining fats, while part of the liver cells in the peripheries are seen to also change into S. D.'s positive staining fats.

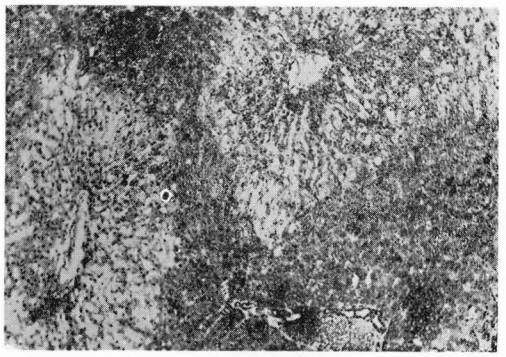

Fig. 5. (No. 57) Necrosis is seen of the liver cells in the central parts of the hepatic lobules by the administration of pyrrol.
H. E. Staining.

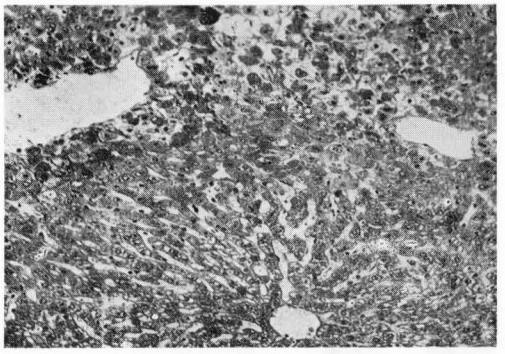

Fig. 6. Liver (No. 73) By the administration of arylformic acid, necrosis is seen in the liver cells in the periphery of the hepatic lobules. H. E. staining.