The Experimental Studies on the So-called Kupffer's Stellate Cell:

Part I.

The Influence of the Indian Ink-injection upon the Stellate Cell and its Relation with the Liver-glycogen.

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I. Introduction and literature.

In 1876, the so-called stellate cell (stern zell), star- or pyramidal shaped, was first described by v. Kupffer.[1] By an application of Gold Chloride to the liver tissue he[2] observed that the stellate cell is an endotheliocyte lying along the blood-capillary of the liver lobule and pocesses a phagocytic power on the disintegrated erythrocytes or various foreign-bodies in the blood circulation. Browicz,[3] Schilling,[4] Schumkowa-Trubina[5] etc. also agreed

with the above view. The vital-staining was first attempted by Heidenhain[6] and later, Chronzezwisky[7] and Wittich[8] applied "carmine" on this subject. It was recently very much progressed by Ribbert,[9] Schlecht[10] etc., and especially by Kiyono[11] and accordingly the stellate cell was also well investigated. By vital-staining with "Lithium-carmine" on rabbit, Sato[12] concluded that the stellate cell which has been hitherto described as an endotheliocyte of the blood capillary is morphologically quite different, and it belongs to that of the adventitia. "It is separated by a membranous mass of the protoplasm from the capillary cavity." "On vital staining, the stellate cell shows relatively coarse granules in its cell-body." "On the contrary, the endotheliocyte of the blood capillary shows very fine granules and its cell-body is enlarged and flattened." "When the endotheliocyte is damaged, the adventitia cell appears among the endotheliocytes, having faced directly to the blood capillary."

"On this occasion, it is frequently enlarged and forms an endotheliocytoid-cell." He also added that on the pathogenic liver, the endotheliocyte of the blood capillary which contains haemoglobin-granules or bile-pigment-granules may be considered to be a transformed stellate cell. Kiyono[11] viewed that the term "stellate cell" is very convenient to be included generically both the histiocytic-sinus-endotheliocyte and adventitia-histiocyte, because these cells belong to the same kinds but on their function there are a little differences between the two. As the stellate cell is a histiocyte, it can live alone and generate. Having considered from the anatomical point of the cell where it faces, on one side, to the blood and on other side, to the liver cell, it may presumably play an important rôle. Kiyono[11] has published a book entitled "Die vitale Karminspeicherung" and described as to the stellate cell in detail. He stated that the stellate cell is a regulator which balances the various products come from the blood to the liver cell and vice versa." "As to its function, it fights strongly against the bacteriotoxin or various poisonous products and not only undamaged by above products but it enlarges and regenerates." "By a stimulation, such an enlarged stellate cell detatches from the wall of the blood capillary and becomes into a histiocyte." "This cell pocesses quite sufficient pigment-granules in it and the total amount of the granules is

obviously increased and at the same time the enlarged granules also can be observed." "When a part of the liver tissue was artificially produced necrosis or on acute or chronic inflammation owing to the action of another etiology, the histiocytic cells assemble to the locality and this phenomenon is performed by the action of the stellate cells, viz., those cells shorten their processes which consist of protoplasms and detatch from the capillary wall." "The stellate cell has actively a phagocytic power in the fixed condition and after it detatches from the capillary wall and becomes a wandering histiocyte, the phagocytic power still more increases and also can take pigment in its cell-body." "The cell in fixed condition is, of course, enlarged and its nucleus is divided into several." Tachaschin[13] proved that the stellate cell which contains pigment-granules can perform the mitosis in both fixed condition or detatched condition. Biondi[14] described that by poisoning of the "Toluylendiamin" there appear iron-pigments in the stellate cells of the liver. Leidemann[15] also proved the similar result on the pernicious anemia. Kato[16] stated that the grade of the hyperglycemia due to an injection of the grape sugar (20% solution of grape sugar 5 c.cm per kilo) is by far inferior to that of the hyperglycemia in the same way but with Indian ink-injection (20 c.c) which was administered six hours ago. "Owing to the reticulo-endotheliocyte, especially the stellate cell is filled up with Indian ink, the anagenetic function on the "histiocyte" is obstructed and consequently the liver cell cannot well manage the superfluous sugar, induced into the blood-vessel, thus he considered that the above matter was resulted. He, therefore viewed that the dysfunction of the reticulo-endotheliocyte is deeply concerned to the function of glycogen-formation in the liver. "On this occasion, the fact that not only the stellate cell but the liver cell also takes the Indian ink in its cell-body cannot be disregarded and this is the point of discussion whether the stellate cell only concerns to the changes of the blood-sugar or not or the liver cell also affects on it." He, however, illustrated the above question by the following reasons:—

1. This experiment was done too early to give sufficient time to take the Indian ink in the liver cell.
2. Even if some amount of Indian ink was taken by the liver cell, it is

too small quantity to be compared with that of the stellate cell, etc.

He concluded that the reticulo-endotheliocyte, especially the stellate cell definitely concerns to the fluctuation of the blood-sugar. Schmidt,[17] Kiyono,[11] Uno,[18] Nishikawa and Takagi[19], etc., have reported that after the spleen was removed, there appears a compensatory-function in the histiocytic cell in the liver. Schmidt[17] experienced that on and after the first day, after the spleen was excised on rat, he proved an iron-reaction in the stellate cell and thenceafter, a few weeks later, the over-growth of the stellate cells form follicular tubercles in the liver. These newly formed cells phagocytose the erythrocytes and also contain pigment-granules which consist of hemoglobin. By resecting the three fourth of the whole liver on rabbit, Yoshinaga[20] observed that the multiplicated stellate cells in the liver lobule have met together and form a cell-column, having connected each other with processes of protoplasms. He viewed that this newly formed tissue is also resulted by a compensatory-phenomenon of the histiocytes to restore the functional defect which have lost by resecting.

II. Methods and materials; in addition, some literature.

My purpose on this experiment is to investigate the relations between the stellate cell and the liver-glycogen. The glycogen is a stored-carbohydrate in animal-body and is very urgent for the anagenesis of its substance. It is out of question that the liver is no doubt a main store-house for it. It is generally acknowledged that the quantity of the glycogen content in the liver increases or decreases after and before meals and its sorts, or excercise or by poisoning of some medicines. The starvation always decreases the liver-glycogen. By Ishimori's[21] quotation in his paper, there have been many written on this subject, on rabbit; viz., Aldehoff[22] observed 0.8% of liver-glycogen and E. Kulz,[25] 0.3 to 0.6% after six days-starvation, however, Pink[23] and Otto[24] reported that there was no more glycogen found in the liver after five and four days-starvation respectively. Ishimori[21] himself observed that the starvation reduces its amount from 0.00

to 0.27% in the liver after four days. Sato[26] considered that there are still some amount of deoxidised substance even the starvation continues more than seven days and it must be a sort of glycogen. My experiment on rabbit proved that the glycogen is slightly traced in the liver after six days-starvation. (rabbit's stomach is always filled with food and even after six days-continuation of starvation it still contains soft excrement-like mass by eating its own excrement and its hair plucked.) The experimental resection of pancreas (or excision of more than 70% of the whole pancreas) increases the sugar content in the blood and at the same time decreases or extinguishes the glycogen content in the liver. Babkin's[27] research showed that the glycogen content of the liver and skeletal muscles in well-nourished rabbits after administration of "Insulin" is markedly lowered as compared with animals fed in the same way but without the injection of insulin. "Insulin always lowers the sugar contents of the blood even after subcutaneous or intravenous injection of very large dosis of glucose (from 25 to 30 gm. in a rabbit weighing two or three kgm.)." "There is no pronounced accumulation of glycogen in the liver and skeletal muscles in rabbits which have received glucose and insulin simultaneously." "Insulin has, apparently, no influence on the glycogen content of the heart." Furthermore, the nourishment, ages and etc., variate the quantity of the liver-glycogen. Prof. Ando[28] described in detail, as to the glycogen on the mammal and the ovipara and he viewed that in the body of chicks-embryo, the liver obtains the function to produce glycogen for the first time at the seventh day after incubation, but the quantity of the glycogen is very little and also very uncertain. Still more, the injection of Adrenalin, phlorizin or Grape-sugar or the "Piqure" can be observed the increasing of the blood-sugar and the decreasing of the liver-glycogen, or the increasing of both the blood-sugar and liver-glycogen. It is quite true to prove glycogen always in the liver of healthy animal. However, as above-mentioned, its quantity is very changeable and so I tried to inject grape-sugar intravenously whenever I want to obtain such animals which heightened both the blood-sugar and liver-glycogen. The grape sugar, extra pure anhydrous, made at Merck & Co., in Germany was always used. Its dosis was made by Fujihara[29] as follows:—

50 gm./dl., of grape-sugar-solution, 10 c.cm. per kilo., and it was injected intravenously from the ear-lobe.

The following table shows a preliminary experiment.

Preliminary Experiment I.

No. of Animal		I	II	III	IV	V	VI
Body-weight (sex) (gm.)		1650 ♂	1720 ♂	1520 ♂	1350 ♂	1620 ♂	1850 ♂
Duration after injection (hour)		1	2	3	4	5	7
Glycosuria	before injection	−	−	−	−	−	−
	after injection	+	+	+	+	+	+
Blood sugar (%)	before injection	0.098	0.095	0.101	0.089	0.093	0.095
	after injection	0.435	0.315	0.180	0.161	0.140	0.109
Liver-glycogen		++	++	+++	+++	++	++

Preliminary Experiment II.

No. of Animal		VII	VIII	IX	X	XI	XII
Body-weight (sex) (gm.)		1450 ♂	1340 ♂	1050 ♂	1180 ♂	1040 ♂	1350 ♂
Duration after injection (hour)		1	2	3	4	5	7
Glycosuria	before injection	−	−	−	−	−	−
	after injection	+	+	+	+	+	±
Blood sugar (%)	before injection	0.091	0.099	0.089	0.091	0.095	0.093
	after injection	0.415	0.305	0.195	0.156	0.135	0.105
Liver-glycogen		++	++	+++	+++	++	++

Preliminary Experiment III.

No. of Animal		I	II	III	IV	V	VI
Body-weight (sex) (gm.)		1890 ♂	1670 ♂	1580 ♂	1350 ♂	1850 ♂	1650 ♂
Duration after injection (hour)		1	2	3	4	5	7
Glycosuria	before injection	−	−	−	−	−	−
	after injection	+	+	+	+	+	+
Blood sugar (%)	before injection	0.091	0.089	0.096	0.095	0.094	0.102
	after injection	0.389	0.291	0.178	0.161	0.126	0.108
Liver-glycogen		++	++	+++	+++	++	++

The average sugar content in the blood of above three groups is as follows:—

Duration after injection (hour)	1	2	3	4	5	7
Blood sugar (%)	0.413	0.303	0.184	0.159	0.134	0.107

The animals were fed with "Tōfukasu," made of beans. The sugar in the urine was examined by Nylander's and Trommer's methods, and the blood-sugar, by Hattori[30] and Kasamatsu's "methylene blue" method. The liver-glycogen was examined by Best's "Carmine" staining and Langhans' "Iodin" method or Saliva-reaction was also applicated. The tissues were partly fixed in 10 per cent solution of Formalin, embedded in paraffin and stained with Alum-hematoxylin-eosin, or Mallory's Eosin-methylene blue; and partly fixed in 95 per cent alcohol, embedded in celloidin and applicated for Best's carmine staining. The section previously treated by Indian ink was stained with hematoxylin alone, so that it can be distinctly differentiated more than that of hematoxylin-eosin. The blood required for sugar-test was obtained by keeping the animal in natural condition without binding it. Stab an ear-vene a little after aseptically cleaned, dried it and selected the naturally dropped-blood, not pressing it. It must avoid to let animals excite or fear, for it affects upon the sugar content in the blood. It has been generally acknowledged that by binding the animal, the hyperglycemia and glycosuria will be resulted. Boehm and Hoffmann[31] described for the first time the above results when they performed tracheotomy upon a cat, having bound it on the operation-table, and they named this phenomenon the "Fesserungs Diabetes." This phenomenon appeared at the 30th minute after operation and continued for about six hours. On this occasion, the blood-sugar is slightly heightened and the liver-glycogen is slightly lowered. Cannon, Shohl and wright[32] called it the "Emotional Glycosuria." Eckhardt,[33] Fujii,[34] etc., also have investigated this subject. On the preliminary experiment, as above showed, it was affirmed that the liver-glycogen is strongly positive in all cases and especially it was maximum at three to four hours after injection. There are many opinions as

to the glycogen-formation in the liver, whether it is performed by the administration of the grape sugar or not, and accordingly the results are also various. As there is a great disparity in quantity of liver-glycogen due to the nourishment and etc., on individual rabbit, having kept the animal preliminarily in starvation, Sato[26] injected the grape sugar solution and observed the aspect of glycogen formation in the liver. He, thus injected the grape sugar intravenously in the ratio of 25 gm/dl., 10 c.cm., per kilo., in rabbits and at about three hours after its administration, the glycogen formation reached to its climax, increasing the liver-glycogen by two to three percent. It was affirmed that this result quite agreed with that of my preliminary experiment which was done in the same way but without starvation and also with different dosis of grape sugar. The Adrenalin chloride (1 : 1000) was next used for the purpose to obtain such an animal whose sugar content in the blood is increased and the quantity of the liver-glycogen is lowered. The solution was injected by Fujiwara[35] in the ratio of 0.4 gm. per kilo., subcutaneously in rabbits. According to his observation, the blood-sugar already increased at an hour after injection; reached its climax at two to three hours and gradually decreased until it returned to normal or nearly normal at 12 hours and entirely to normal at 24 hours. Several literature relates to hyperglycemia due to the adrenalin-administration will be here added. In 1901, Blum[36] first observed the temporary glycosuria by subcutaneous injection of extractum of suprarenal capsule, in dogs or rabbits. Pollack[37] noticed that by subcutaneous injection of adrenalin chloride, the blood-sugar increases by 0.27, 0.37 and 0.42 per cent. By exstirpation of suprarenal capsule in rats, Schwarz[38] observed the pronounced decreasing of liver-glycogen. Kahn and Starkenstein[39] also viewed the similar result in rats but it was quite normal in rabbits, treated in the same way. Porges[40] also viewed the similar result in dogs as that of the above rats. Wolownik, Doyon and Kareff[41] stated that the appearance of sugar, caused by an adrenalin chloride-injection must come mostly from the liver-glycogen. Falta and Priestly[42] found that the quantity of the blood-sugar, occurred by adrenalin chloride-injection, after binding the all blood-vessels flow into the liver, accounted only for 0.04 per cent, but it increased by 0.27 to 0.35 per cent by adrenalin-chloride-injection without

binding the blood-vessels. They believed that such hyperglycemia and glycosuria due to the adrenalin-injection are caused by the reduction of liver-glycogen and the glycogen of muscles does not concern to. Iwano[43] reported that by adrenalin chloride-injection in dogs, the muscle-glycogen is markedly decreased in spite of the liver-glycogen is not so much affected and on the contrary, by exstirpation of pancreas, it is vice versa. As above mentioned, it is generally acknowledged that the adrenalin chloride-injection causes the reduction of liver-glycogen. My experiment has shown that the adrenalin chloride-injection has apparently an influence on the liver-glycogen but it is not so distinct. Still, the animals were kept in starvation for five to six days, to diminish the glycogen content in the liver and etc., and by this treatment, the experimental animals could be kept nearly in the same condition upon the quantity of the liver-glycogen. A trace of glycogen, left in the liver, on this occasion, can be disregarded. As previously described, the stellate cells actively phagocytose various foreign-bodies in the blood-circulation and applying this function, the Indian ink was injected intravenously. By this application the relations between the liver-glycogen and the stellate cell were investigated.

Indian ink:—

A good "Sumi" principally made of soot and some gelatin was rubbed with normal saline solution on an ink-stone. It was then centrifugated, filtrated and sterilized by heating. When in use, having kept in body-temperature, the fresh solution was injected intravenously 10 c.cm per kilo., in rabbits. This dosis does not hurt the animal but sometimes causes to lose the body-weight slightly. It is very hard to obtain a standard density of the solution and so the "Sumi" was rubbed and cetrifugated for five minutes each to make 50 c.cm. of this solution. This injection must be done very slowly and carefully as the rapid-injection sometimes causes rapid death or after a few hours it may die. It also must not be contained any air-bubble in the solution as it is black in color, and hardly find out such bubble.

III. Experiments.

Experiment one :—

In three well-nourished rabbits, the grape sugar-solution, 50 gm./dl., 10 c.cm., per kilo., were injected intravenously and three hous later, cases 1 and 2 were treated with Indian ink-injection, 10 c.cm., per kilo., and sacrificed after an hour. Case 3, the control was similarly treated but without Indian ink-injection and was sacrificed. The result is shown in Table I.

Table I.

No. of case		I	II	III
No. of animal (sex)		16 ♂	20 ♀	36 ♀
Body-weight (gm.)		1520	2355	2640
Glycosuria	before injection	−	−	−
	3 hours after grape-suger-injection	+	+	+
	1 hour after Indian-ink-injection	+	+	
Blood-sugar (%)	before injection	0.060	0.101	0.096
	3 hours after grape-sugar-injection	0.170	0.137	0.150
	1 hour after Indian-ink-injection	0.247	0.244	
Liver-glycogen		++	++	+++

Glycosuria indicates with +, − only (positive and negative)
Liver-glycogen indicates with +, −
± means slightly positive
+ positive ++ relatively strong positive;
+++ strong positive and − means negative.

The histologic findings of the liver :—

Cases 1 and 2. It has passed an hour only after the Indian ink was injected and the stellate cells are already filled with the fine granules of Indian-ink. The granules are very fine and very sparsely filled in the cell-body. The stellate cells are usually spindle or star in shape and the nuclei are surrounded with fine granules of Indian ink and no enlarged cells are

seen. A few of the nuclei are sometimes enlarged or consisted of two. These stellate cells mainly occupy the wall of the blood-capillary and a few are freely wandering in the capillary. The granules of Indian ink also freely exist along the wall of the blood-capillary and so the latter is seen very distinctly, having been divided with minute black lines. Several leucocytes phagocytosed Indian ink-granules are also seen in large and small blood-vessels. The glycogen in the liver is relatively strong positive (+ +) by Best's carmine staining.

Case 3 (control). The shape of the stellate cell is obscure, as it is not treated with Indian ink. Its nucleus is mostly round or spindle in shape and some are consisted of two. There are relatively many wandering stellate cells in the capillary and moreover, some of them phagocytose erythrocytes. The glycogen in the liver is strong positive (+ + +).

Experiment two:—

In this experiment, the Indian ink was injected in three rabbits in almost the same ages and cases 1 and 2, after 24 hours and case 3, after an hour were respectively sacrificed. The Table II (a) shows it.

Table II (a).

No. of case		I	II	III
No. of animal (sex)		12 ♂	25 ♂	17 ♂
Body-weight (gm.)		1430	1430	1530
Glycosuria	before injection	−	−	−
	an hour after Indian-ink-injection		−	−
	24 hours after Indian-ink-injection	−		
Blood-sugar (%)	before injection	0.107	0.102	0.065
	an hour after Indian-ink-injection			0.108
	24 hours after Indian-ink-injection	0.105	0.096	
Liver-glycogen		+	++	++

The histologic findings of the liver:

Cases 1 and 2. The granules of Indian ink relatively markedly invade

the stellate cell-body and in some of them, these granules intermingle each other and so such a cell is appeared as a black mass. These stellate cells enlarged by filling so many granules and wandering in the capillary are relatively many found. Some of the nuclei are enlarged and some are consisted of two. These cells as above mentioned are not so many and generally they are mostly spindle in shape and the nuclei are surrounded with fine granules of Indian ink. Some of the cells are enlarged and form caverns which are surrounded with black granules or irregular networks. There are also some stellate cells which take erythrocytes in their cell-bodies. The liver-cells and the epithelial cells of the bile-duct are normally existed. The glycogen presents in positive (+) in case 1 and relatively strong positive (++) in case 2.

Case 3. The granules of Indian ink are finer and sparsely filled in the stellate cells than those of the previous cases and such a black mass, formed by intermingling of the granules is not seen in this case. Some of the cells are enlarged and wandering in the capillary, being detatched from the wall of the capillary. Several leucocytes which contain some granules of

Table II (b).

No. of case		I	II	III
No. of animal (sex)		49 ♂	50 ♀	51 ♀
Body-weight (gm.)		2660	2310	2225
Blood sugar (%)	before injection	0.093	0.075	0.105
	30 minutes after Indian ink-injection		0.117	0.112
	an hour after „ „	0.108	0.091	0.109
	two hours after „ „		0 097	0.104
	three hours after „ „	0.115		
	four hours after „ „		0.092	0.084
	five hours after „ „	0.101		
	six hours after „ „			0.075
	eight hours after „ „	0.076		
	24 hours after „ „		0.088	0.086

Indian ink are also seen in the small blood-vessels. The liver is slightly hyperemic and the rest of the liver tissue is quite normal. The glycogen is in similar to that of case 2.

In addition to the above experiment, the Indian ink were injected in three rabbits and the sugar content in blood in various hours was estimated and the Table II (b) shows it.

By this experiment, it was affirmed that the Indian ink injected in the body heightens the quantity of the blood-sugar for about four to five hours and after six hours it always returns to its normal content.

Experiment three: —

Four rabbits were kept in starvation for six days. By this treatment, the disparity of the quantity of liver-glycogen in all cases generally could be lessened. Cases 1 and 2 were sacrificed after above treatment had done, and case 3 was injected the Indian ink after six days-starvation and sacrificed after an hour. In case 4, the Indian ink-injection was repeated once every day, (5 c.cm.) during the starvation period, and sacrificed 24 hours later after the last injection had done. It is shown in Table III (a).

Table III (a).

No. of case		I	II	III	IV
No. of animal (sex)		19 ♂	40 ♂	18 ♂	24 ♂
Body-weight (gm.)	before starvation	1380	1265	1350	1620
	after starvation	1050	835	1075	1265
Glycosuria	before starvation	—	—	—	—
	after starvation	—	—	—	repeated-injections of Indian-ink
	an hour after Indian ink-injection			—	—
Blood-sugar (%)	before starvation	0.094	0.102	0.095	0.096
	after starvation	0.072	0.084	0.085	
	an hour after Indian ink-injection			0.105	0.055
Liver-glycogen		±	±	—	—

In addition, the Table III (b) shows the result of sugar contents in blood and urine in various hours, caused by Indian ink-injection after six days-starvation.

Table III (b).

No. of case		I	II	III	IV	V
No. of animal (sex)		39 ♂	41 ♂	43 ♀	44 ♀	42 ♂
Body-weight (gm.)	before starvation	1070	1275	1345	1195	1260
	after starvation	780	1090	1110	965	1000
Glycosuria	before injection	—	—	—	—	—
	after starvation	—	—	—	—	—
	an hour after Indian ink-injection	—				—
	2 hours after „ „		—	—	—	
	6 hours after „ „			—		
	24 hours after „ „					—
Blood-sugar (%)	before injection	0.088	0.071	0.070	0.085	0.098
	after starvation	0.043	0.051	0.050	0.067	0.051
	an hour after Indian ink-injection	0.051				0.057
	2 hours after „ „		0.069	0.052	0.072	
	6 hours after „ „			0.040		
	24 hours after „ „					0.040

The histologic findings of the liver are as follows:—The result of glycogen in Table III (b), however, cannot be estimated, as it is cited from Experiment five.

No. 19. As it was not injected the Indian ink, the shape of the stellate cell is obscure. Its nucleus is mainly spindle in shape and occupies the wall of the blood-capillary. No wandering stellate cells are seen but a little. The liver is generally hyperemic. The glycogen in the liver shows only a trace (±).

No. 18. In this case, the Indian ink was only once injected and examined an hour later, and so the granules of the Indian ink are very

fine and are sparsely filled in the stellate cells and enlarged one can scarecely be seen. The free-existing fine granules of the Indian ink are also seen mainly along the wall of the capillary. The leucocytes contained the granules of the Indian ink are wandering in some dilated blood-capillary and not in other blood-vessels. The liver is slightly hyperemic and the rest is quite in normal. In this case, even a trace of glycogen cannot be detected in the liver (−).

No. 24. This one was repeated the Indian ink-injection for six days and so the stellate cells are markedly saturated with the granules of the Indian ink and represented as enormously enlarged black masses. Some still keeps its regular shape, but its nucleus is scarecely seen in it. Some flattened nuclei are also seen arround the black masses, having been pressed by the latter. There are also quite many fine granules of the Indian ink freely in the blood-capillary. The leucocytes, contained the above granules are slightly detected. The glycogen in the liver is absolutely negative (−).

Discussion of above three experiments:—

Summarizing the above three experiments, it will be here discussed. Practically, as shown in the preliminary experiment, the sugar content in the blood is in the highest if it is examined immediately after the grape sugar was administered, and is gradually lowered until it returns to normal content. The fact in cases 1 and 2 of Experiment one is quite different. In spite of the blood-sugar in these cases, three hours later after injecting the grape sugar shows 0.117 and 0.137 respectively, an hour later after injecting the Indian ink, the blood-sugar in the same cases estimates 0.247 and 0.244 respectively (viz., four hours later after the sugar-injection). Such an irregular phenomenon was caused by the Indian ink. Moreover, by Best's carmine staining, the liver-cell in case 3, the control is stained deeper than those of cases 1 and 2. Considered from these facts that the injected grape sugar is partly transformed into the glycogen in various organs and tissues besides liver; partly discharged from the kidneys, and moreover, is remained in the liver and skeletal muscles etc., without changing, though it cannot make a sweeping statement, at least in this experiment, it may say that the above described hyperglycemia in cases 1 and 2 must have come from the reduced sugar of the liver-glycogen which was

once formed by the sugar-injection; and moreover some sugar which was still remained a little in the blood by the same injection. The reason why the liver-glycogen once formed by the sugar-injection is as above mentioned reduced cannot be illustrated without the Indian ink. Still, supposing that the Indian ink directly does not affect the skeletal muscle-glycogen nor invade the muscle tissue, but that the stellate cells in the liver phagocytose the granules of the Indian ink immediately after its injection, it may presumably concluded that the stellate cell, Indian ink and the liver-glycogen are closely connected each other.

In Experiment two, the Table II (a) shows that an hour later after injecting the Indian ink, the blood-sugar is slightly heightened and at the same time, the liver-glycogen is a little lessened than that of nomal one which was not previously treated with Indian ink. This fact is also presumed that the Indian ink-injection must be closely concerned to the liver-glycogen and compared with that of previous experiment, the grade of the sugar content in the blood was not so distinct in this case. This may be considered that the former was followed with sugar-injection besides Indian ink-injection and the latter, with Indian ink-injection but without the sugar. Still, in this experiment, it was found that 24 hours later after injecting the Indian ink, the blood-sugar is slightly lowered or nearly same to that of normal one and this result seems to stand against the above hypothesis, but the Table II (b) apparently proves that if the blood-sugar in cases 1 and 2 had estimated an hour later after injecting the Indian ink, as that of case 3, a slight increasing of blood-sugar would be also observed. Table II (b) affirmed, that the blood-sugar in normal rabbits is slightly increased for about four to five hours after injecting the Indian ink. By Experiment two, as above illustrated, it was confirmed that in early period after the Indian ink-injection, the liver-glycogen is reduced and accordingly the blood-sugar slightly heightened but in later period the liver-glycogen does not reduce at all directly by the Indian ink and accordingly the blood-sugar remains in normal.

In Experiment three, the following result was observed that, by starvation, the blood-sugar as well as the liver-glycogen is markedly lowered and especially the latter will be finally diminished to a trace. It has al-

ready been described in Chapter II, that there are many opinions as to the liver-glycogen in starved animal. Still, in some animal, it is said that the liver-glycogen has no influence on the starvation but it is suddenly diminished at the time of death. Some believe that by starvation, the liver-glycogen is gradually lowered but it is heightened temporarily just before death. It is, however, generally believed that by starvation, the liver-glycogen is gradually diminished until it is completely expired. In my experiment, the liver-glycogen is always diminished to a trace by six days-starvation. This is quite righteous as a result of normal anagenetic function, viz., the stored carbohydrate, especially the liver-glycogen is gradually reduced for the insufficient nutrition due to the starvation. As above mentioned, the liver-glycogen is markedly reduced by six days-starvation, but it is always remained a trace. However, it could not be found even a trace of glycogen in the liver in case 3 of Table III (a) which was followed by Indian ink-injection after it was starved for six days, and sacrificed an hour later. At the same time, the blood-sugar in this case is slightly heightened. This fact also agrees with the above hypothesis that the Indian ink is definitely concerned to the liver-glycogen. Still, the case 4 in Table III (a) was repeated six times to inject Indian ink 5 c.cm every day, and sacrificed 24 hours later after the last-injection. On this occasion, there was no more glycogen detected in the liver. This is also quite righteous and affords no scope for discussion. Furthermore, Table III (b) shows that the blood-sugar is slightly heightened for a few hours after injecting the Indian ink on starvation but after six hours it is vice versa. In this Table III (b) though the result of liver-glycogen cannot be observed, as it was cited from Experiment V and also it was used for further-investigation but supposing from the result of Table III (a), it may be considered that the liver-glycogen has been completely expired. This fact still confirms the presumption of Experiment II, though this one was tested after starvation.

Summarizing the above three experiments, it may be considered that the Indian ink, stellate cell and the liver-glycogen have closely connected with each other. It was already described in Chapter I that the stellate cell is a regulator which balances the various products, come from the blood

to the liver-cell and vice versa, and consequently it may definitely say that the stellate cell plays an important rôle for a regulator between the blood-sugar and the liver-glycogen. Considered the above experimental result, applying this hypothesis, it will come to the following conclusion: namely, the Indian ink induced into the blood-circulation abnormally stimulates the stellate cell and consequently the latter causes a regulative dysfunction and this loses the balances between the liver-glycogen and the blood-sugar. Thus the glycogen in the liver is gradually resulted its reduction as above experiment shows. This regulative dysfunction in the stellate cell, however, does not continue so long but it is gradually adapted itself to the abnormal stimulation due to the Indian ink and after a certain period, it is restored to the normal function, therefore the existence of the Indian ink in the stellate cell in later period is affected no longer to the normal function of the stellate cell.

Experiment four:—

Three of rabbits were equally lessened the disparity of the quantity of liver-glycogen, having kept them in starvation for five days. In cases 1 and 2, the Indian ink was injected once every day continuously for three days after starvation and well fed after the sixth day. In the evening of the seventh day (viz., 12 hours later after the last Indian ink-injection) the grape sugar was first injected and twice more continued in the morning and afternoon on the eighth day, then sacrificed three hours later after the last sugar-injection.

Case 3, the control was also treated in similar way as in cases 1 and 2 but without the Indian ink-injection and having well fed during this period, sacrificed three hours later after the last sugar-injection. It is shown in Table IV.

The histologic findings of the liver:—

In cases 1 and 2, the granules of the Indian ink in the stellate cell are apparently increased more than that of the stellate cell which was treated once with Indian ink and the granules form a black mass in the cell, having coalescented. The stellate cell is generally enlarged and its nucleus occupies the center of the cell-body or is pressed out to the periphery or

Table IV.

No. of case		I	II	III
No. of animal (sex)		22 ♂	27 ♂	26 ♂
Body-weight (gm.)	before starvation	2480	1370	1410
	after starvation	2000	1095	1110
	at sacrifice time	2220	1190	1230
Glycosuria	before injection	−	−	−
	3 hours after the 3rd sugar-injection	+	+	+
Blood-sugar (%)	before injection	0.084	0.109	0.101
	3 hours after the 3rd sugar-injection	0.170	0.195	0.170
Liver-glycogen		++	++	++

some cannot be detected. Others are spindle or star in shape. The stellate cell which is wandering in the capillary is usually enlarged. The rest is in normal.

In case 3, the control, the stellate cell-body is obscure as it was not treated with Indian ink. Its nucleus is generally round or spindle in shape and some are enlarged. These cells are occupied along the wall of the blood-capillary and some are wandering.

The liver-glycogen in all cases is equally relatively strong positive (++).

Discussion of Experiment four:—

In this experiment, the repeated injections of the Indian ink as well as those of the grape-sugar were apparently superfluous. It was at first feared that, after starvation, the almost all of glycogens in the body are markedly decreased and so the repeated injections of the grape sugar are necessary to meet all requires in the body and moreover to spare room on glycogen-formation in the liver etc., and accordingly, if the injection of Indian ink is insufficient, this experiment will not afford a satisfactory result. Later, by examining the following Experiment five, it was, however, understood that not only the above treatment was quite an imaginary fear but such repeated-injections complicate the result. In this experiment, the

repeated-injections of Indian ink undoubtedly should expire the tolal glycogen in the liver. On this occasion, the fate of the first and second injections of the grape sugar cannot be detected, and the third injection is very urgent in this experiment. Three hours later after the last sugar-injection, viz., at the maximal period on glycogen formation in the liver, it was sacrificed. The liver-glycogen is relatively strong positive (++) in both cases 1 and 2 and at the same time, the blood-sugar-contents are nearly similar to that of case 3, the control. In other words, in cases 1 and 2, the third injection of the grape sugar was tried 30 hours later after the third injection of the Indian ink had done, namely when the stellate cell was already adapted itself to the abnormal stimulation due to the Indian ink and restored to its normal function, the third sugar-injection was done, and so the sugar, induced into the blood-circulation at this time, caused the above result quite similarly as in case 3, the control. In this experiment, the stellate cells in cases 1 and 2 as well as the control, case 3 had carried on their normal functions, unconcerned to the existence of the Indian ink.

Experiment five :—

In this experiment, nine rabbits were kept in starvation for six days and then they were studied in various ways as follows. The Table V (a) shows it.

Case 1. Three hours later after the sugar was injected sacrificed-

Case 2. An hour later after injecting the Indian ink, the sugar was followed and three hours later sacrificed-

Case 3. 24 hours later after injecting the Indian ink, the sugar was followed and three hours later sacrificed-

Case 4. Two hours later after the Indian ink-injection, the sugar was followed and three hours later sacrificed-

Case 5. Six hours later after injecting the Indian ink, the sugar was followed and three hours later sacrificed-

Case 6. Two hours later after injecting the Indian ink, the sugar was followed and two hours later sacrificed-

Table V (a).

No. of case		I	II	III	IV	V	VI
No. of animal (sex)		38 ♂	39 ♂	42 ♂	41 ♂	43 ♀	44 ♀
Body-weight (gm.)	before starvation	1060	1170	1260	1275	1345	1195
	after starvation	730	780	1000	1090	1110	965
Glycosuria	before starvation	−	−	−	−	−	−
	after starvation	−	−	−	−	−	−
	an hour after Indian-ink-injection		−	−			
	2 hours after „ „				−	−	−
	6 hours after „ „					−	
	24 hours after „ „			−			
	an hour after grape-sugar-injection				+	+	+
	2 hours after „ „						+
	3 hours after „ „	+	+	+	+	+	
Blood-sugar (%)	before starvation	0.085	0.088	0.098	0.071	0.071	0.085
	after starvation	0.048	0.043	0.051	0.051	0.050	0.067
	an hour after Indian-Ink-injection		0.051	0.057			
	2 hours after „ „				0.069	0.052	0.072
	6 hours after „ „					0.040	
	24 hours after „ „			0.040			
	an hour after grape-sugar-injection				0.295	0.371	0.419
	2 hours after „ „						0.265
	3 hours after „ „	0.120	0.080	0.063	0.065	0.079	
Liver-glycogen		++	±	++	+	++	±

Still, three additional cases were studied as follows. The Table V (b) shows it.

Case 7. An hour later after the sugar was injected, sacrificed-

Case 8. Two hours later after the sugar was injected, sacrificed-

Case 9. An hour later after injecting the Indian ink, the sugar was followed and two hours later sacrificed-

Table V (b).

No. of case		VII	VIII	IX
No. of animal (sex)		46 ♂	48 ♂	47 ♂
Body-weight (gm.)	before starvation	1280	1525	1475
	after starvation	1055	1295	1095
Glycosuria	before injection	−	−	−
	after starvation	−	−	−
	an hour after Indian-ink-injection			−
	an hour after sugar-injection	+	+	+
	2 hours after „ „		+	+
Blood-sugar	before injection	0.091	0.090	0.080
	after starvation	0.053	0.069	0.045
	an hour after Indian-ink-injection			0.047
	an hour after sugar-injection	0.320	0.385	0.413
	2 hours after „ „		0.251	0.160
Liver-glycogen		+	++	−

The histologic findings of the liver:—

Case 1. As no Indian ink was administered, the shape of the stellate cell is quite obscure, but its number seems to be slightly increased. Its nucleus is generally spindle in shape and some are enlarged. The glycogen is not accumulated evenly in the liver-lobules and by Best's staining, the centrum of the lobule is stained deeper than the periphery and its grade is relatively strong positive (+ +).

Case 2. The fine granules of the Indian ink are sparsely existed in the stellate cell-body. The stellate cell is mostly spindle or star in shape and can scarecely seen an enlarged one. The glycogen in the liver is detected as only a trace (±).

Case 3. As it has passed 24 hours after the Indian ink-injection, there are many enlarged stellate cells contained black masses which consisted of granules of the Indian ink. Even if such a black mass was not formed, the each granules are very coarse. There are also a few stellate cells in spindle

or star in shape. The free-existing granules of Indian ink are also seen in the blood-capillary. The glycogen is relatively strong positive (+ +).

Case 4. Although there are quite many granules of the Indian ink in the stellate cells, each granule is very fine and is not coalescented each other. The stellate cell is generally spindle or star in shape and the nucleus located the center of the cell-body is distinctly seen. There are also found several enlarged and wandering stellate cells and stillmore a few free-existing granules of Indian ink in the blood-capillary. The glycogen in the liver-cells is positive (+).

Case 5. The granules of the Indian ink in the stellate cells are somewhat remakably seen. Some of the granules existed in the wandering stellate cells are coalesced each other and such cells are slightly enlarged and still, their nuclei are sometimes hardly detected. The glycogen in the liver is relatively strong positive (+ +).

Case 6. This one is nearly resemble to that of case 4, however, the glycogen is scarcely detected in the liver-cells (±).

The result of the additional cases is as follows:—

Case 7. This one is in similar condition to that of case 1, but the liver-glycogen stains irregularly and spottedly by Best's method (+).

Case 8. This is also similar to that of the previous one but the liver-glycogen is relatively strong positive (+ +).

Case 9. The granules of Indian ink in the stellate cell are relatively numerous and surrounded the nucleus but never coalescented each other. The cell is usually star or spindle in shape. The free-existing granules of Indian ink are slightly seen in the blood-capillary. The glycogen in the liver is absolutely negative (−).

Discussion of Experiment five.

This experiment supplies the defficiency of Experiment four and further-more illustrates the relations between the stellate cell and the liver-glycogen in Experiments one, two and three.

In case 1, it is out of question that the glycogen in the liver will be nearly disappeared by six days-starvation. On this occasion, however, the fact that the glycogen was proved in relatively strong positive (+ +) by the sugar-injection followed has swept off such a doubt as mentioned in Experiment four, (namely only one time of sugar-injection is insufficient to form glycogen in the liver in such a starved animal). The quantities of liver-glycogen and the blood-sugar at this time are quite lower than those

of the preliminary experiment and this will be well illustrated by the reason that the former was treated in well-nourished rabbit and the latter, in starved one.

In case 2, it is apparently true that there will be no more glycogen existed in the liver after the Indian ink was injected, having considered from the result of Experiment three. At this time, the stellate cell is to be abnormally stimulated by the Indian ink and caused a regulative dysfunction for some period. If this is true, how is the fate of the grape sugar induced into the blood-circulation? Were the glycogen proved in the liver, case 2 would be apparently comflicted with the above hypothesis. However, the fact that the glycogen was not formed in the liver but a trace tells us that the above hypothesis is righteous. Thus, not only the stellate cell in regulative dysfunction reduces the glycogen of the liver but it also disturbs the glycogen formation in the liver. In this case, however, a trace of glycogen was detected in the liver and this will be illustrated by the reasons that at sacrificed time, it has already passed four hours after the Indian ink was injected and so the regulative dysfunction in the stellate cell due to the abnormal stimulation of the Indian ink is nearly going to restore its normal function and that the still remained sugar in the blood is of use on the glycogen formation in the liver and that such a period is very short and the quantity of such sugar is also very little.

In case 3, six days-starvation and Indian ink-injection completely expire the glycogen content in the liver. This is of useless to illustrate. It has passed 24 hours after the Indian ink was injected and so the stellate cell is, at this time, quite adapted itself to the stimulation of the Indian ink and it is to be performing the normal function. The sugar induced, on this occasion, therefore, must be taken part in glycogen formation in the liver and the existence of the Indian ink does not make any difference on this function. The fact that case 3 showed glycogen in relatively strong positive (+ +) in the liver quite agrees with the above view. The quantity of the liver-glycogen, at this time is lower than that of the preliminary experiment and this also causes from such a reason as illustrated in case 1.

Case 4. The glycogen in the liver was positive (+) .and this one was sacrificed five hours later after the Indian ink was injected. In early period, the sugar induced into the blood-circulation cannot be taken up by the liver, as the stellate cell; the regulater is in regulative dysfunction.

In later period, however, the sugar still remained in the blood is gradually received by the liver, as the stellate cell is, at this time restored to its normal function. Thus, the glycogen was formed in the liver, but as it was very short time, the result was not so distinct.

Case 5. This one was examined six hours later after injecting the Indian ink and next the sugar respectively. This resulted the liver-glycogen in relatively strong positive (+ +) as in cases 1 and 3. It is unnecessary to illustrate, having considered from the above cases.

Case 6. It has passed four hours after the Indian ink and next the sugar were respectively injected and the glycogen was not detected in the liver but a trace as in case 2. This is also righteous by such a reason as illustrated in case 2 and cannot take exception at all.

Case 7. This one was tried to study, on the starvation, how long will it take to form glycogen in the liver after the grape sugar was injected. It was examined, in this case, an hour later after the sugar was injected. The result was positive (+) but by Best's method, it scatterly stained, one part deeply and the other lightly, and so it cannot say that an hour-application is quite sufficient to form glycogen in the liver.

Case 8. In this case, the liver-glycogen was observed in relatively strong positive (+ +), two hours later after the sugar was injected. This tells us that two hours duration is quite sufficient to form glycogen in the liver.

Case 9. In this case, the Indian ink was injected and an hour later, the grape sugar-injection was followed and two hours later, sacrificed. The glycogen at this time was absolutely negative (−) in the liver and this also cannot be denied.

By above various experiments, it was well learnt that the abnormal stimulation due to the Indian ink has an influence on the stellate cell. However, here is a question as follow remained which must be solved.

On Indian ink-injection, the Indian ink itself existed abundantly in the blood-circulation may have an influence on the liver-glycogen and in later period, when the Indian ink was completely expired from the blood-vessels, then it does not concern to the liver-glycogen and consequently, the grape sugar induced into the blood-circulation may for the first time take

its part on glycogen-formation in the liver. In fact, the Indian ink induced into the blood-circulation, is very quickly swept up from the blood-vessels and only five minutes later after its injection, mainly the stellate cells in the liver; sinus-venosus-endotheliocytes, reticular-cells and splenocytes in the spleen; and sinus-venosus-endotheliocytes and reticular-cells in the bone-marrow are already invaded from the fine granules of the Indian ink. Besides these cells, the free-existing granules of Indian ink are also seen slightly in the blood-plasma and lympho-apparatus. This matter furthermore increase the above doubt. Although the Indian ink is so quickly swept out from the main blood-vessels, the fact that the blood-capillaries in the liver-lobules still contain many free-existing granules of the Indian ink, even 24 hours later after its injection cannot be considered that the existence of Indian ink in the blood-vessels only may have an influence on the liver-glycogen. Still, the fact that the blood in the capillary only is always in an intimate connection with various organs and tissues and not directly with those of large and small blood-vessels, even there are no more Indian ink in the latters, on this occasion, the existence of the Indian ink in the blood-capillaries of the liver-lobules will be able to dissolve the above doubt. Stillmore, the fact that the glycogen content in the skeletal muscles which decreased by starvation, is increased by the grape sugar-injection without any connection with the existence of the Indian ink, indorses that the above view is righteous.

Experiment six : —

This experiment seems to be superfluous and unnecessary, considering from the previous experiment, but there are a little difference between the two, namely, the former was observed on starved animal and the latter, on well-nourished animal.

Two well-nourished rabbits were treated with Indian ink-injection and 24 hours later, the sugar-injection was followed and three hours later sacrificed. For control, No. 36 was cited here. In this experiment, it must be noticed that the quantity of the blood-sugar is quite different, compared with that of the previous experiment. In the previous experiment, the cases which were treated with both Indian ink- and grape sugar-injections

always showed hyperglycemia for two hours after the latter was injected, but at the third hour, the blood-sugar was lowered to the normal or under-normal, in spite of case 1 which was treated with grape sugar-injection only still showed hyperglycemia. In this experiment, the blood-sugar at the third hour after injecting the grape sugar was very high and nearly same or a little lower than that of the control and also the preliminary experiment. This is the point of discussion. By starvation, the stored carbohydrate in the body is enormously consumed and so the grape sugar induced into the blood-circulation is harriedly required from the various organs and tissues to meet a need, and consequently, such sugar will be quickly expired. This cannot be disregarded for one of urgent reasons. However, as case 1 in Experiment five shows, if the Indian ink was not previously injected, the grape sugar is still high even three hours later after the grape sugar was administered and this result opposes the above view. My experiment is limited to a few cases and also Sato's experiment is very indeterminate; when he observed the blood-sugar three hours later after the grape sugar (25 gm./dl. 10 c.cm. per kilo.) was injected on four days-starvation it sometimes showed hyperglycemia and sometimes decreased more than the blood-sugar which was examined just after starvation. Considering from these results, it cannot be definitely concluded, but at least by above experiment, it may be able to say that the Indian ink induced into the blood-circulation on starvation has an influence upon the glycogen content in the blood and it seems to cause the grape sugar expire quicker than that of cases which were not previously administered the Indian ink. This experiment, however, must be furthermore investigated. The histologic findings in the liver is much alike to those of the previous cases which were tested 24 hours later after injecting the Indian ink and so it will not be here described. Table VI illustrates it. The liver-glycogen in cases 1 and 2 is relatively strong positive (+ +) and a slightly weaker than that of case 3; the control.

Discussion of Experiment six:—

In this experiment, it has passed 24 hours since the Indian ink was injected and so the stellate cell in the liver is, at this time, to be already restored its normal function, having adapted itself to the abnormal stimu-

Table VI.

No. of case		I	II	III	IV	V
No. of animal (sex)		11 ♂	14 ♂	15 ♂	35 ♀	36 ♀
Body-weight (gm.)		2190	1525	1350	2660	2640
Glycosuria	before injection	−	−	−	−	−
	24 hours after Indian-ink-injection	−	−	−	−	
	3 hours after sugar-injection	+	+	+	+	+
Blood-sugar (%)	before injection	0.105	0.096	0.096	0.069	0.077
	24 hours after Indian-ink-injection	0.073	0.070	0.068	0.067	
	3 hours after sugar-injection	0.180	0.122	0.152	0.147	0.120
Liver-glycogen		++	++	++	++	+++

lation due to the Indian ink and accordingly on the grape sugar induced, on this occasion, the stellate cell should perform its fundamental function, thus, in cases 1 and 2, the glycogen was apparently formed in the liver as in case 3, the control. It is also righteous that the blood-sugar in cases 1 and 2 is must alike but the liver-glycogen is slightly lower than that of case 3, because the former was treated previously with the Indian ink and this matter caused slight reduction of the liver-glycogen in early period.

Experiment seven:

It has been described in Chapter II that, in rabbit, the adrenalin chloride causes the hyperglycemia as well as glycosuria and at the same time reduces the liver-glycogen. In fact by adrenalin-injection, the hyperglycemia occurs but the liver-glycogen is not so distinctly reduced, so this experiment referred to the adrenalin-injection is of no interest on the subject of this paper. Here is added only a few cases and it is shown in Table VII.

Case 1 was treated with 1000 : 1 solution of adrenalin chloride 0.4 gm. per kilo., subcutaneously and the blood-sugar was observed after 3, 15 and 18 hours respectively. At the third hour, hyperglycemia was proved but at the 15th and 18th hour, it was restored to normal, and the liver-glycogen

Table VII.

No. of case		I	No. of case		I	II
No. of animal (sex)		28 ♂	No. of animal (sex)		32 ♂	33 ♂
Body-weight (gm.)	before injection	1285	Body-weight (gm.)		1565	1470
	after injection	1230	Glycosuria	before injection	−	−
Glycosuria	before injection	−		24 hours after Indian-ink & adrenalin-injection	+	
	3 hours after adrenalin-injection	+		3 hours after adrenalin-injection		+
	15 hours after „ „	−	Blood-sugar (%)	before injection	0.072	0.092
	18 hours after „ „	−		24 hours after Indian-ink & adrenalin-injection	0.207	
Blood-sugar (%)	before injection	0.108		3 hours after adrenalin-injection		0.322
	3 hours after adrenalin-injection	0.226	Liver-glycogen		+	++
	15 hours after „ „	0.089				
	18 hours after „ „	0.088				
Liver-glycogen		++				

was, at the 18th hour, relatively strong positive (+ +).

In case 2, the Indian ink was injected and 21 hours later, the adrenalin chloride-injection was followed and three hours later it was sacrificed.

Case 3 was similarly treated with adrenalin chloride as in previous case but without the administration of Indian ink. Compared with the above two cases, the blood-sugar was higher in the latter than the former and at the same time, the liver-glycogen was positive (+) in the former and relatively strong positive (+ +) in the latter. The above differences between the two, should have come from the reason that the former was previously treated with the Indian ink and the latter, without it and accordingly the former caused a slight reduction of live-glycogen by the Indian ink in early period. It is too imprudent to conclude with such a few cases and must be furthermore investigated.

Experimeut eight:

Kato investigated the hyperglycemia due to the injection of the grape sugar and the other hyperglycemia occurred in the same way but with

Indian ink-injection which was previously administered. This has been described in Chapter I and here, the same experiment was added and accordingly, both the Indian ink and the grape sugar used are estimated 10 c.cm. per kilo., and 20% sol. of grape sugar 5 c.cm. per kilo., respectively. Thus the blood-sugar was observed and compared with that of Kato's view. Table VIII shows it.

Table VIII.

No. of case		I	II	III	IV	V
No. of animal (sex)		52 ♂	53 ♂	54 ♂	55 ♂	56 ♂
Body-weight (gm.)		1290	1695	1210	2280	1680
Quantity of Indian ink and its duration after injection (hour)		0	10 c cm. per kilo.	〃	〃	〃
		0	1	6	6	24
Quantity of grape sugar		20% sol. 5 c.cm. per kilo.	〃	〃	〃	〃
Blood-sugar (%)	before injection	0.090	0.102	0.082	0.073	0.077
	5 minutes after sngar-injection	0.451	0.593	0.343	0.364	0.479
	30 minutes after 〃	0.239	0.358	0.174	0.198	0.213
	1 hour after 〃	0.100	0.267	0.094	0.084	0.137
	2 hours after 〃	0.082	0.128	0.061	0.083	0.077

Case 1. The grape sugar only was administered in a rabbit.

Case 2. The grape sugar was followed an hour later after the Indian ink was injected.

Cases 3 and 4. The grape sugar was followed six hours later after the Indian ink was injected.

Case 5. The grape sugar was followed 24 hours later after the Indian ink was injected.

Discussion of Experiment eight:—

These five cases are not sufficient to investigate the subject, but according to the above various experiments, even the quantity of the grape sugar used is different from the others, the result shown in Table VIII is definitely righteous.

Five minutes later after the grape sugar-injection, the sugar content

in the blood was enormously high in all cases, above all, case 2 stand in the highest; cases 1 and 5, in middle; and cases 3 and 4, in the lowest. Case 5 was previously treated with Indian ink and it had passed 24 hours when the grape sugar was injected, so the stellate cell is, at this time, no more affected to the Indian ink and accordingly there should be no differences between this case and case 1 which was similarly treated with the grape sugar but without Indian ink-administration. Thus, the blood-sugar in these two cases was quite similar. The grape sugar used in both cases will be accepted a little by the liver.

In case 2, the grape sugar was administered after the Indian ink had injected just an hour ago and consequently, the stellate cell is extremely in the condition of regulative dysfunction. Thus, the grape sugar induced at this time, into the blood-circulation cannot be accepted from the liver and so it is righteous that the blood-sugar is in the highest among them.

In cases 3 and 4, it has passed six hours after the Indian ink-injection and this tells that the stellate cell is mostly restored to its fundamental function. Still, the liver, at this time, is slightly in want of the sugar as the liver-glycogen was reduced a little by the Indian ink-injection and had no time to restore it. Thus, the grape sugar induced into the blood-circulation should be used in the liver more than that of cases 1 and 5. This fact resulted the above hyperglycemia.

Kato's observation is quite different. The blood-sugar examined five minutes later after it was treated with both the Indian ink- and grape sugar-injections as in cases 3 and 4 of this experiment was measured average 0.6396. On the contrary, the blood-sugar, examined five minutes later after it was treated with the grape sugar-injection alone as in case 1 of this experiment was measured average 0.2894. Thus, the blood-sugar of the former was measured nearly two and two tenth times larger than that of the latter. The blood-sugar in my cases is as above mentioned, nearly in similar grade at this time even there is a little disparity. Only five minutes have passed since the grape suger was injected and in such a short time, this grape sugar will not be able to concern in the glycogen-formation of the liver, muscles etc., and it is nothing but a little even it concerns to. The existence of the Indian ink at this time, therefore, does

not make any difference on the live-glycogen. Considering from this view, the results of above five cases are quite reasonable and so cannot agree with Kato's view. After 30 minutes, in cases 1 and 5, the blood-sugar was gradually lowered and at the first hour after the grape sugar was injected, it nearly returned to the normal and at the second hour, completely to the normal. Case 2 still showed hyperglycemia at the second hour and it is always higher than the others.

In Cases 3 and 4, the blood-sugar was lowered to the normal at the first hour. These results are also reasonable. Kato's observation rather agrees with case 2 which was previously treated with Indian ink an hour ago and not with cases 2 and 3 which were treated in same way as Kato's cases. Still, many experiments were tried by Kato to investigate various changes in the blood-sugar caused by injecting Indian ink or "Toluylendiamin" which reacts on the function of the reticuloendotheliocyte. He could not well illustrated by his experiment only that why the blood-sugar is heightened when the stellate cell is stimulated by "Toluylendiamin" and why it is lowered when the stellate cell is interfered its function, by being filled with Indian ink. However, considering that such changes in the blood sugar cannot be presumed to have come by only the changes of the hemolysic-function in the endotheliocyte, especially the stellate cell, he concluded that the characteristic function of the stellate cell should have a definite relation to the blood-sugar. Considering from my conclusion, it cannot be always affirmed the point of his view that the stellate cell filled with Indian ink causes an obstruction on the anagenetic function of the histiocyte. It is also advisable that, on this occasion, the liver-glycogen must be examined so far as the "Toluylendiamin" has particularly a function to stimulate the stellate cell. It is also very requisite to examine it in various hours after the Indian ink was injected and still in starvation. These things would cause the different results and certainly be more interested on this subject.

IV. Summary and conclusions.

It was well proved by many experiments that in early period, the stellate cell causes a regulative dysfunction by the Indian ink-injection and this has an influence on the reduction of the liver-glycogen. Furthermore, from the opposite side, it was observed that after the liver-glycogen was entirely expired, how the Indian ink, induced into the blood-circulation will play on the grape sugar which was followed and it was concluded that the regulative dysfunction of the stellate cell also interferes the glycogen-formation of the liver. It was stillmore observed that the stellate cell in a regulative dysfunction which caused by the Indian ink as above mentioned is gradually adapted itself to the abnormal stimulation due to the Indian ink and certain period later, it restores to the normal function. It was thus proved by many experiments that the grape sugar, induced, on this occasion, into the blood-circulation will act normally on the glycogen-formation in accordance with the requisite of the animal.

Conclusions:—

I. The Indian ink, induced into the blood-circulation, in early period, abnormally stimulates the stellate cell in the liver and this causes a regulative dysfunction of the stellate cell.

II. The regulative dysfunction of the stellate cell due to the abnormal stimulation of the Indian ink is not permanent and the stellate cell is gradually adapted itself to such abnormal stimulation and after a certain period, it restores to the normal function and therefore, such regulative dysfunction is nothing but a temporary reaction.

In concluding, I wish to express my thanks to Prof. Dr. Tamura who kindly gave me this subject and also sincerely appreciate his suggestive and stimulating criticism which has made this work possible.

(Completed on November 15th 1924)

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