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Histochemical Studies of Lipids.

I. Histochemical Examination of Gaucher's Disease.

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Introduction.

It is commonly said that, Gaucher's disease has a tendency to fall in a family in a group. In this disease, spleen and liver get gradually big until these organs attain a gigantic form, while superficial lymph nodes do not markedly swell. Typical enlarged cells of this disease appear in hematopoietic organs, i.e. spleen, liver, bone-marrow and lymph nodes, but not on other organs such as thymus, lungs, brain, suprarenal bodies etc., where similar enlarged cells of Niemann-Pick's disease do in occur. Then cytoplasm of Gaucher's cells appear homogeneous or to be composed of extremely fine foamy appearance, and seldom shows coarse foams like that of Niemann-Pick's cells. But these characteristics of Gaucher's disease are not always distinctly recognizable. There are several cases, as already reported by Dienst(1), Hamperl(2), Oberling and Woringer(3), in which the progress of the disease, such as rapid enlargement of spleen in infantile period, and other clinical as well as pathological manifestation suggest the disease as that of Niemann-Pick's, yet the usual histochemical examination and chemical analysis point to as Gaucher's. Accordingly, at present, the writer believes in that a definite diagnosis of Gaucher's disease should be given only when a rich content of kerasin can be detected in the enlarged cells. Owing to the fact, however, that there is no trustworthy method of examining kerasin in tissue cells, it has commonly been thought to be very difficult to demonstrate.

Five years ago, prof. Okamoto and his collaborators, among them was the writer, reported a series of new histochemical methods by means of which cerebroside and other lipids can be identified separatedly(4)(5)(6)(7). Applying these methods the present research was undertaken to examine fatty substances contained in Gaucher's cells and to study the relation between these substances and the processes of this disease as well as its pathohistological changes.

Materials Examined.

The writer have had occasions to examine six cases of Gaucher's disease of which four were Japanese and the other two Americans. All of them are summarised in the following table (see Table 1). Nos. 2, 3, 4 and 5 were diagnosed as

Gaucher's disease by clinical and pathological aspects. Nos. 1 and 6 were clinically diagnosed as Niemann-Pick's disease, but according to pathological examinations they were thought to be Gaucher's and this was confirmed by the writer's examination.

Table 1. Summary of materials examined.

Case	Age and sex	Invasion	Duration	Family relation	Diagnosis and reporter	Pathological findings	N.B.
No. 1	6 months male	Soon after birth	6 months	—	Niemann-Pick's D. or Gaucher's D.	Hyperplasia of Gaucher's cells in spleen, liver, suprarenin and thymus etc.	Diagnosed clinically as Niemann-Pick's D., but as Gaucher's by microscopical & chemical examination.
No. 2	10 months	?	?	?	Gaucher's D.	Hyperplasia of Gaucher's cells in spleen, liver, suprarenin and lymph nodes.	
No. 3	1 year & 9 months	9 months after birth	1 year	—	Gaucher's D.	Hyperplasia of Gaucher's cells in spleen, liver, lymph nodes, thymus, bone-marrow and lungs etc.	
No. 4	adult male	?	?	?	Gaucher's D. & tuberculosis of lungs.	?	
No. 5	?	?	?	?	Gaucher's D.	?	
No. 6	?	?	?	?	Niemann-Pick's D.	?	Diagnosed pathologically as Gaucher's disease.

Methods of examination adopted in the present research.

A small pieces of several organs are embedded in paraffin and also in gelatine, and sections are stained with hematoxylin and eosin, and also with van Gieson's staining for ordinary histological examination. Lipids contents of Gaucher's cell are examined by means of our special histochemical methods, often accompanied by Sudan III and Schultze's method of cholesterin-fat, when necessary, iron-staining. Our special methods were already reported by Okamoto, the others and the writer himself in Japanese (4) (5) (6) (7). But as it has not been widely known, the procedures are outlined.

I. The staining methods of fatty acids and their salts (by K. Okamoto, M. Ueda and A. Kato)(4).

A. The common test method.

1. The preparation of frozen section of organs or tissues that are fixed with newly prepared 10% neutral formalin, that is saturated with NaCl (more than 20%).

2. Thorough washing of the section with over 20% NaCl solution.

3. (a) Section is put into the following solution for 24 hours at a room temperature.

Distilled water.....100 cc.
Saturated aqueous solution of cupric nitrate $Cu(NO_3)_2 \cdot H_2O$.....0.4~0.5 cc.

or, (b) Put the section into the following solution for 2 ~ 3 hours or longer at room temperature.

75% Ethyl alcohol.....100 cc.
Saturated aqueous solution of cupric nitrate.....0.2 cc.

Caution : Different sections must be used for (a) and (b).

4. Thorough washing of the section.

5. Put the section into the following solution, and keep it for 24 hours at 50 ~ 60°C.

95% ~ 100% Ethyl alcohol.....100 cc.
Saturated alcoholic solution of p-dimethyl-aminobenzylidene-rhodanine.....5 cc.

Caution : Sodium acetate may be added to the above solution at the rate of 2%.

6. Washing.

7. Staining with hematoxylin and glycerin enclosure.

Findings : (1) By this method, fatty acids and their salts (oleic acid, stearic acid, palmitic acid and Na-,K-, Ca- and Mg- salts of them etc.) show reddish brown, which are the result of copper-p-dimethyl-aminobenzyliden-rhodanine reaction (salts, which are formed with fatty acid and iron, do not shown this reaction).

(2) Various fatty acids and their salts have different manners of reactions by the kinds of solvents which dissolve cupric nitrate. Accordingly the writer adopted at same time both methods of 3. (a) and 3. (b) methods. Using these two methods, differentiations of all fatty acids and their salts could be done. When Na- and K-soap, or Ca- and Mg- soap of oleic acid, and palmitic acid exist in the specimen, 3. (b) method of the treatments mentioned above, was omitted, however, if there are no such substances, 3. (a) method of that treatments was omitted.

II. The staining method of cholesterin (by K. Okamoto, H. Shimamoto and H. Sonoda)(5).

A. Sulphuric acid iodine tincture method.

1. Fixation with formalin and preparation of frozen section.

2. Taking the section on the slide and remove water thoroughly with filter paper and drop the following solution upon it.

{ Conc. H_2SO_4 ..... 10~12 cc.
{ Dil. iodine tincture ..... 20 cc.

Caution: (1) This solution can not be preserved for long time, even when it is closely covered.

(2) Diluted iodine tincture is prepared by diluting iodine tincture (iodine 6.5 g., potassium iodide 2.5 g., 95% alcohol 91 cc.) with equal volume of 95% alcohol.

3. After setting the coverglass, examine under microscope.

Findings: Cholesterin appears green or blue green.

Caution: This preparation can not be kept for long time. It should be examined within 2 ~ 3 hours preferably within 5 ~ 10 minutes.

III. The staining methods of phospholipids (by K. Okamoto, T. Shimamoto, M. Ueda, Y. Kusumoto and D. Shibata)(6).

A. The common test method.

1. Fixation with formalin and preparation of the frozen section as thin as possible.

2. Put the section into the following solution for 48 hours in a room temperature.

{ Acetone ..... 15 cc.
{ Magnesium chloride ..... 0.1 g.

3. Thorough rinsing of the section.

4. Put the section into the following solution for 24 hours in an ice-chamber.

{ 0.2% Sodium chloride solution ..... 0.2 cc.
{ Saturated solution of mercuric nitrate with 60% alcohol ..... 10 cc.

5. Washing thoroughly.

6. Put into potassium iodide solution for 4~5 minutes.

Caution: Soon after the section is thrown into that solution, the color of section changes to yellow or slightly reddish yellow. But after several minutes, the color of section begins to fade and after 2~3 minutes, it disappears completely.

7. Washing of the section for 10 minutes.

8. Put into 2% sodium acetate solution for 10 minutes.

9. Put the section for 10 minutes into the saturated solution of diphenylcarbazone with 80~100% alcohol.

10. Thorough washing of the section.

11. Glycerine enclosure.

Finding: Phosopholipids appears to bluish violet.

Cautions: (1) By this method, cerebroside shows positive result too. Accordingly we must do detection of phospholipids and cerebroside as follows; after washing of the section, put into pyridine for 48 hours in an ice-chamber. If the

lipid is phospholipid, the section will turn positive but when cerebroside, the reaction disappears.

(2) Some other materials beside lipids, show positive result by this method. In such cases, we must detect phospholipids by following treatment; if the section do not show negative result by the method, mentioned above, it is thrown into pyridine for 3 days in room temperature after that or is warmed in it for 1 hour at 55°C, this materials are not lipids.

B. The separation test method.

Method (a).

1. Fixation with formalin and preparation of the frozen section.
2. Rinsing of the section with acetone in order to take off water.
3. Put the section into ether for 2 days at room temperature.
4. Thoroughly remove the remaining ether from section with acetone.
5. Apply the treatments from 3 to 11 of the common method A.

Finding: By this method, sphingomyelin alone shows positive result.

Method (b).

1. Do the treatments from 1 to 4 of the common method A.
2. Remove water thoroughly from section with acetone.
3. Put the section into ether for 2 days.
4. After rinsing the section for a time, apply all treatments from 5 to 11 of the common method A.

Finding: By this method, sphingomyelin and lecithin show positive result.

(c) When some lipid shows negative result by common method A, this lipid is likely to be cephalin.

IV. The test method of cerebroside (by K. Okamoto, M. Ueda, Y. Kusumoto and M. Hashimoto)(7).

1. Fixation with formalin and preparation of frozen section (20~30μ).

2. The section is to be treated with cold acetone in an ice-chamber for a period of 2 days, or section placed on the slide, after thoroughly dried up, should be immersed in the following mixture at 4~6°C for 1~2 hours.

Ether	1 volume
95% alcohol	4 volume

3. After drying up the section on the slide which has been warmed at 75°C previously, 1~2 drops of the following solution that has been warmed at 75°C is dropped upon it in order to be acted at 75°C for 70~90 secunds.

5% α-Naphthol solution in 95~100% alcohol	0.1 cc.
Conc. H_2SO_4 (Sp. G. 1.84)	2.0 cc.

Caution : The solution could be kept for 2~3 days long, but it is advisable to prepare solution freshly at use. Conc. H_2SO_4 must be pure.

4. Put the coverglass and examine microscopically.

Finding : Cerebroside take beautiful purplish-red color. The color changes, at first, light red, then purplish-red to dark-purple or gray and then finally vanishes. Therefore, microscopic examination should be made soon after preparation and examine once again after 30 or 60 minutes in order to avoid being overlooked.

In order to examine whether or not above mentioned methods give correct results, each of following substances was injected subcutaneously on the experimental animals, and then a piece of the tissue of that part, and sometimes the lymph nodes corresponding to that parts, too, was dissected, on which our methods were applied and was confirmed applicability.

Fatty acids; pure palmitic, stearic and oleic acids supplied by Merk, in Germany.

Cholesterin ; pure crystall supplied by Merk.

Salts of fatty acids, lecithin, cephalin, sphingomyelin and cerebroside were given with kindness of Prof. Ueno of Osaka University, who is one of the foremost specialists in organic chemistry, especially of fat chemistry. He prepared phospholipids and cerebroside from human brain, and confirmed purity of these substances by definite examination methods.

Results.

I. Distribution of the Gaucher's cell-like specific enlarged cells.

In cases of Nos. 1, 2, 3 and 4 mentioned above, almost all organs in the body were examined histologically, while at Nos. 5 and 6, only a small piece of spleen was obtained, so that the researches of the specific enlarged cells at various organs except spleen were done on the former 4 cases.

Results are summarized in Table 2.

Details observed in each organs are described as follows.

(1) Spleen : All six samples give a similar figure. Several or sometimes more than twenty of enlarged cells gather to a group like a cell-nest, thus forming a node, which is surrounded by a few number of small lymphocytes, fine connective tissue fibers and endothelial cells making the wall of compressed venous sinuses. Sometimes the enlarged cells lie in a row around small vessels to form sheath, or solitary cell is scattered about in the pulp-cords or in the venous sinuses.

The nodes of the enlarged cells seem to be placed in the pulp-cord but they are so densely placed almost all over the spleen that the cords and lymphatic follicles are pressed and the ordinary structure of the spleen as well as they ordinary

Table 2. Distribution of the enlarged cells.

Case	Age and sex	Spleen	Liver	Lungs	Heart	Kidneys	Pancreas	Aorta	Lymph nodes					Adrenal glands	Tonsils	Lymphoid apparatus of intestinal wall
									Cervical nodes	Paratracheal nodes	Nodes in hilus of lungs	Nodes in the head of pancreas	Mesenteric nodes			
No. 1	6 months male	###	##	–	–	–	–	–		##	##		###		###	##
No. 2	10 months	###	##	–	–	–	–	–		###		###		##		
No. 3	1 year and 9 months	###	##	##	–	–	–	–	##	###	+	###	##			
No. 4	Adult male	###	##	–	–	–	–	–		###		###				
No. 5	?	###														
No. 6	?	####														

position of central arteries are scarcely observable.

Most of enlarged cells appear polygonal, spherical, oval or ellipsoidal, and a few fusiform. Their diameter measures 25~35 microns, except in the sample of No. 3, where rather small cells of about 15 microns or so, as well as extremely large mononuclear cells of about 50~60 microns are also observed.

The cytoplasm of usual enlarged cells seems to be homogeneous on frozen sections, and on paraffin sections, except that the sample of No. 3 contains finely granulated cells observed on paraffin sections, and also several 3 or 4 nuclear gigantic cells. Again, in the samples of Nos. 3 and 4, are observed several enlarged cells containing brown pigments of which iron reaction is negative.

Findings of lipids staining: The enlarged cells are generally stained faintly by Sudan III, and appear light orange, except the finely granular cells found in the sample of No. 3 mentioned above, which is not stained by Sudan III.

According to examinations by our new methods of lipids staining, it is confirmed that the enlarged cells of all cases examined contain a large quantity of cerebroside, and also cholesterin-fat and lecithin in a greater or smaller amount, while sphingomyelin is not detected distinctly.

These lipids mentioned, especially cerebroside are only found in the enlarged cells, but they are not detected in ordinary tissue cells of spleen.

(2) Liver: Specimens of Nos. 1, 2 and 4 show typical sign of liver cirrhosis, with nodes of the enlarged cells formed in the interstices, but smaller nodes are also seen in pseudolobuli, which are usually composed of a few or several enlarged

Table 3. Results of lipids staining of enlarged cells of spleen.

Case	Age & sex	Neutral fat	Fatty acid	Cholesterin fat	Cholesterin	Phospholipids			Cerebroside
						Lecithin	Cephalin	Sphingomyelin	
No. 1	6 months male	−	−	++	−	±	−	±	+++
No. 2	10 months	−	±	++	−	+	−	±	+++
No. 3	1 year & 9 months	±	−	+	−	+	±	±	+++
No. 4	Adult male	−	−	±	−	+	−	−	+++
No. 5	?	−	−	±	−	+	−	−	++
No. 6	?	−	−	±	−	±	−	−	++

cells. The enlarged cells in pseudolobuli present themselves in a variety of form.

In the tissue of No. 3, in which no sign of liver cirrhosis is observed the enlarged cells are intermixed among atrophic liver cells. These two types of the cells closely resemble with each other in shape, so that the morphological differentiation is not easily done.

Findings of lipids staining: Most of the enlarged cells are faintly stained by Sudan III, while there are a few cells which are stained by this dye intensively. The lipids thus stained are proved to be composed of a small quantity of cholesterin-fat and lecithin, while existence of sphingomyelin is uncertain. Cerebroside is not detectable on the stellate cells or other interstitial cells as well as ordinary liver cells, though they have fallen into atrophy.

(3) Lymph nodes: In lymph nodes of Nos. 1, 2 and 4, intense changes are observed, contents of lymphatic follicles are displaced for most part by nodes of the enlarged cells, while lymphocytes remain around the follicles only in narrow zones. Lymph sinuses disappear as consequence of pressure due to development of the nodes. A part of endothelial cells of lymph sinuses swell up in spindle form and then changing to the enlarged cells. Often we meet with those sinuses in which round enlarged cells alone are observed.

Most of the enlarged cells are polygonal or round and about 15~30 microns in diameter, while there may happen to see large ones of some 50 microns or so, or those of large size containing 3 or 4 nuclei. In the case of No. 3, pathological change of lymph nodes can be classified into two divisions. The one, which is represented by the lymph nodes in the head of pancreas, is quite similar to those of afore mentioned (*see* Fig. 1).

The other is seen on lymph nodes found in hilus of lungs, on mesenteric as

well as cervical nodes, where a few enlarged cells are observed in lymphatic follicles, which cells communicate by cytoplasmic processes with enlarged cells or reticulo-endothelial cells exist around them (*see* Fig. 2), while endothelial intersinus cells of lymph sinuses show no change.

Fig. 1. The lymph node in the head of pancreas, where many enlarged cells are observed.

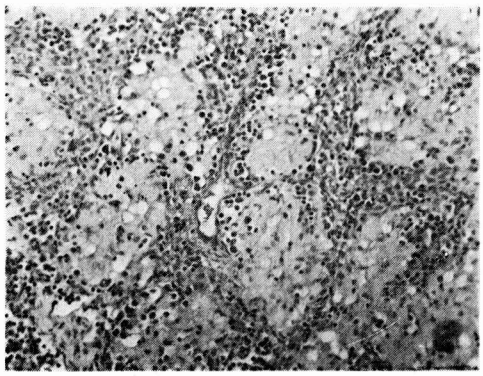
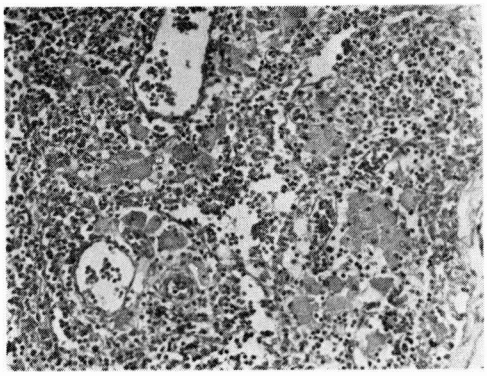


Fig. 2. The lymph node in hilus of lung, where a few enlarged cells are observed.



Cytoplasm of the enlarged cells looks like homogeneous hyaline substance in paraffin sections except those slightly affected found in the specimen No. 3, of which the cytoplasm often appears finely foamy.

Findings of lipids staining: All enlarged cells contained lipids, among which cerebroside fills a greater part, though existence of cholesterin-fat, lecithin and sphingomyelin are also confirmed. In other cells of the lymph nodes no such lipids are detectable.

(4) Adrenal glands: In general the development of adrenal cortex is not good, while that of the medulla is much better, in which the enlarged cells are scattered. These cells appear in various shapes, e.g. fusiform, spherical, polygonal etc., and also in various size.

Findings of lipids staining: Contents of lipids in cortical cell are relatively poor, although cholesterin-fat and phospholipids are detected but no cerebroside is recognizable. The enlarged cells are found in the medulla, which are stained by Sudan III in a light orange color, and their lipids content is rich cerebroside and poor in lecithin.

(5) Tonsils: In interstitial tissues of lymphatic follicles in tonsils are scattered many enlarged cells, so that the follicles are compressed in a certain degree. The enlarged cells are mostly polygonal. There are also seen a few enlarged cells scattered inside the follicles. The enlarged cells are proved to contain a large amount of cerebroside and less of cholesterin-fat and lecithin.

(6) Lymphoid apparatus of the intestinal wall: The enlarged cells are mainly seen in the lymphoid nodules of appendix and in the Peyer's patches of ileo-coelical

part, though in a far less intensity than liver, spleen or lymph nodes. They are mostly found individually among many lymphocytes. Their shape are mostly polygonal in size of 20 microns or so; while a few of large cells of about 50 microns or so are also observed. They contain a moderate quantity of cerebroside and a small amount of cholesterin-fat and lecithin.

(7) Lungs: In the majority of the lungs (in Nos. 1, 3 and 4), there is observed scene of inflammation, i.e. infiltration of miscellaneous cells which make the morphological detection of the enlarged cells almost impossible. By means of our lipids staining, however, the enlarged cells containing cerebroside intermixed with lecithin and cholesterin-fat are detected in the specimen of No. 3 alone. In this case the mentioned cells are seen around small vessels in a few numbers mostly with a shape of spheroid.

(8) Other organs:

Kidneys: No enlarged cells, which contain cerebroside could be seen.

Pancreas: Glandular cells show atrophy in a light degree, but no enlarged cells to be found.

Aorta: In specimens of Nos. 1 and 3, slight thickening of inner coat is observed, without any deposition of cerebroside and other lipids.

Discussion.

At present, Gaucher's disease may be defined as a condition in which a certain organs such as spleen and liver, etc. are charged with a large number of typical enlarged cells, and at the same time content of cerebroside, especially of kerasin is proved to be considerably augmented as chemical analysis has shown. It is generally supposed that the cerebroside thus increased is definitely contained in the enlarged cells; but as far as the writer knows, no one has ever proved it directly hitherto, as there has been no histochemical method of detecting cerebroside in the cell body under microscope. Nevertheless, by means of our histochemical methods cited in this report, this direct verification made possible, only in those cases, when the enlarged cells have been proven by this method to contain cerebroside should be diagnosed as Gaucher's disease.

As a result of the present examination, it is confirmed that the enlarged cells found in spleen or other organs of six cases contain a large quantity of cerebroside, and a relatively small amount of lecithin, cholesterin-fat and sometimes of sphingomyelin.

Three of our cases (Nos. 1, 2 and 3) are infantile cases, in which the disease progressed rather rapidly until they die. In these cases, the typical enlarged cells have found to be distributed, not only in spleen and liver, which are usual in Gaucher's

disease, but also in the adrenal gland, lungs, tonsils and lymphoid apparatus of intestines. Such a wide distribution of enlarged cells rather suggest Niemann-Pick's disease than Gaucher's, though in our cases extensive distribution to skull bone or superficial lymph nodes, were not seen, as is often observed in the former disease. In short, the distribution of the typical enlarged cells resembles to Gaucher's disease of infantile period, reported by Hamperl (2).

According to Epstein and others (8), fresh Gaucher's cell has a homogeneous cytoplasm, which shows a foamy structure containing fine fibrous substances by Mallory's staining. In the present cases, where paraffine sections were stained by hematoxylin and eosin, most of Gaucher's cells show a homogeneous scene, while several of No. 3 case seems finely foamy. The reason for such a foamy scene cannot be attributed to the fact that the lipids contained in the cell have been dissolved out by alcohol, or other fat soluble solvents used in preparing section, as all specimens were treated in the same way.

Most of Gaucher's cells observed in our specimens are spherical or polygonal and of 30~40 microns in diameter, while a few of them appear as megalocytes containing 3~4 nuclei.

The origin of Gaucher's cells has been discussed by Gaucher himself and other authorities (8), but at present, it appears that the consensus of opinions would be that these cells are originated from reticulo-endothelial cells. The following findings of the present research also support this opinion. In lymph nodes, in which the affection seems rather slight, the enlargement of the cell begins from the reticular cells found in the follicles, which the cells are pushing out processes of cytoplasm and make communication with processes of intact reticular cells surrounding them in the follicle. In a lymph node, in which a large amount of mature enlarged cells are seen, those cells lose their processes and appear polygonal, and assemble to make a node, yet, characteristic properties or reticulo-endothelial cells are still retained. Even in a far advanced case, typical change of the endothelial cells of lymphsinuses could scarcely be seen.

As already stated, Gaucher's cell is charged with a considerable quantity of cerebroside and a less amount of lecithin and cholesterin-fat. Existence of these lipids is limited to Gaucher's cells alone, but are not found in the surrounding connective tissue cells or epithelial cells ; even in tissue cells of adrenal glands and fatty tissues, which are rich in fat, these special lipids are not found.

As stated above, Gaucher's cells are stained by Sudan III faintly red. This is understood by the fact that the cells contains a small amount of lecithin and cholesterin-fat, which have a certain affinit to Sudan III. In such a case where the enlarged cell contains, beside cerebroside, relatively large amount of lecithin or cholesterin-fat, these cells are stained intensively by Sudan III, and may be diagnosed

as Niemann-Pick's disease by mistake. In such a case, it will be difficult to differentiate Gaucher's disease from Niemann-Pick's other than resorting to the aid of our histochemical differentiation method.

The author believes that this histochemical method is quite useful in differentiating Gaucher's disease from other diseases of similar nature, because of its superiority over other methods in its value of diagnostic aid and simplicity of procedure.

Summary.

(1) Six pathological specimens, which came from patients clinically diagnosed as Gaucher's or Niemann-Pick's disease were examined. They were diagnosed as Gaucher's disease, by the reason of large cells presented which contained a large amount of cerebroside, a small quantity of cholesterin-fat and lecithin, and also sphingomyelin in some cases.

(2) Cerebroside was found only in the enlarged cells called Gaucher's cell and not found at all in other tissue cells.

(3) In the three infantile cases examined, Gaucher's cells distributed fairly widely, in several organs, and were not confined to spleen, liver and lymph nodes, as it has been generally believed. Kinds of lipids contained in Gaucher's cell of infantile cases were quite the same as the one of adult case, which contained a large quantity of cerebroside, cholesterin-fat, lecithin and sphingomyelin etc.

(4) It was concluded that Gaucher's cells come from reticulo-endothelial cells especially from reticular cells of spleen or lymphatic follicles. In an early stage of this change, young Gaucher's cell communicates with intact reticular cells surrounding it with cytoplasmic processes. Later it take a polygonal or round from loosing its processes.

(5) Though the cytoplasm of Gaucher's cells is homogeneous in some cases, and in other cases, it is faintly foamy, substances involved in cells are almost the same. It should be born in mind that, when paraffin section is prepared, cerebroside contained in these cells might have dissolved entirely into solvents applied.

(6) By the current method of histological lipids-staining, it is impossible to confirm the existence of cerebroside in the cells, because by Sudan III stain, cerebroside will not be stained, and also by Smith-Dieatrich's staining the differentiation of cerebroside from phospholipid is impossible. In order to give difinite diagnosis of Gaucher's disease, it is necessary to prove the existence of cerebroside in the tissue by the method of chemical analysis, or to detect the existence of the same substance in the typical enlarged cells by means of this histochemical methods of cerebroside, which will be superior to the others in its practice.

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