A Study upon Hypothalamo-hypophysial Neurosecretion in Experimental Diabetic Rabbit and in Sulfonylurea Administered Rabbit.

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Since the experiments of depancreatic diabetes by O. MERING and J. V. MINKOWSKI (1889), the trend of investigation into diabetic metabolisms, symptoms, complications and therapies, using experimental diabetic animals appeared, and thereafter, many experimental diabetes by using various animals were succeeded.

At first, it was alloxan diabetes which was pursued by H. R. JACOBS (1937). And after them, L. C. DUNN (1943) found out the typical changes of blood sugar level after alloxan administration and concurrently discovered selectively damage of beta cells in the islets. This study stimulated C. C. BAILEY and O. T. BAILEY (1948) and they succeeded in making continued hyperglycemia avoiding fatal hypoglycemia by glucose solution injection. Furthermore, recently, OKAMOTO et al., (1949) discovered that another substances, dithizone, oxine and so on have the same effect as alloxan, and these drugs cause continued hyperglycemia, destroying beta cells by combining their zinc.

On the other hand, therapy of diabetes made great progress since the discovery of insulin by BANTING and BEST (1922), and by the improvement of insulin, therapy of diabetes has become simpler and surer than what it was. But with all the superiority of insulin, however, the therapeutic effect appeared only by injection, and this was the only and greatest weak point of insulin.

So many internal medicines for diabetic therapy were studied to remedy this fault, but many of them were forgotten without receiving recognition of their faculties.

The most important observation of recent antidiabetic internal medicine might be the accidental finding of sulfonylurea such as IPTD, BZ 55 and D 860 which were found out one after another in France and Germany, and their effectiveness was also recognized; then, their clinical data were reported.

There has been made many experiments of sulfonylurea, but the mechanism of this drug remains still undecided. As it said above, there has been repeated many experiments upon experimental diabetes and the mechanism of sulfonylurea; but they were chiefly done upon metabolism or histology of the pancreas, and there were a few experiments upon hypothalamo-hypophysial system which has a close relation to polyuria, the essential symptom of diabetes. Namely, E. KRATZ-
SCH (1951) reported the reduction of GOMORIophile substances in posterior pituitary of alloxan rat, and further more, FUJITA et al. (1957) reported the changing of hypothalamic neurosecretions cell and also he inferred the relation between antidiuretin and polyuria of an experimental diabetic dog.

But, there has been no report of neurosecretion on each stage, from hypoglycemic shock after alloxan administration to continued hyperglycemic stage. So, I investigated the hypothalamo-hypophysial neurosecretion on each stadium in alloxan diabetic rabbits and simultaneously observed the neurosecretion in sulfonylurea administrated rabbits, just to investigate the effect of the drug to the neurosecretion.

I. Material and Method.

White healthy 35 rabbits, weighing about 2 kg, were used and 5 of them were used as the control. And 15 of them were administrated alloxan, another 15 of them administrated sulfonylurea.

0.5 % solution of EASTMAN KODAK's alloxan was injected into ear vein of rabbits, then, 4—6 hours after, the first hypoglycemic shock attacked the rabbits, and then, it repeated several times; at each attacks, 20 % of glucose solution was injected to the rabbits' vein preventing shock death.

Blood sugar level was measured by HAGEDORN JENSEN's method, 24 hours after alloxan administration for the first time, then each three days, and at the hyperglycemic stadium, it was tested once a week.

On the other hand, about the sulfonylurea; a short and little bamboo stick was set between rabbit's teeth and NELATON's soft rubber catheter (No. 6) was put into stomach through the bamboo. And 20 cc suspension of BZ 55 or D 860 was administrated through the catheter.

There I made 2 groups of sulfonylurea administrated rabbits, one group were administrated sulfonylurea 0.25 g/kg only once, and the other group were continuously administrated the same doses for 10 days.

Materials were taken as follows:

Lobotomy was done carefully after slaughter by air embolism and hypophysis was removed as it was connected with interbrain, and then, immediately put into ZENKER's fixative solution. Next, hypophysis with interbrain was inbedded by methylbenzoat celloidin paraffin, and cut into 6 μ section, then stained by GOMORI's CHP method.

II. Experimental Results.

a) Stadium of alloxan hypoglycemic shock.

In 4—7 hours after the intravenous administration of alloxan 150 mg/kg, the first shock attacked the rabbit, and glucose solution was given to prevent the shock death, then, 24 hours later, blood sugar level showed over 200 mg/dl. Some of them recoverd to normal blood sugar level within several days, but about half of them showed continued hyperglycemy. There were few objective finding but increase of urinal quantity and decrease of body weight upon these experimental
By the GOMORI's staining, observation upon normal hypothalamic neurosecretions nuclei, nucleus paraventricularis and nucleus supraopticus of normal rabbit were as follows:

Mostly, the neurosecretions cells were round or oval, and many of them were biaxle and axles dispositions were generally irregular. The nucleus was round and

Fig. 1. Nucleus supraopticus of the normal rabbit. 6 μ. GOMORI’s staining. There are full of GOMORIphile granules in the cytoplasm, the nucleus is light and a nucleolus is clearly observed. 4,000×

Fig. 2. Posterior pituitary of the normal rabbit. 6 μ. GOMORI’s staining. There are much GOMORIphile substances in posterior pituitary but they always be apt to gather especially in the peripheral part of posterior lobe, and here and there, some of HERRING’s bodys lie scattered. And GOMORIphile substances are also observed at surrounding of the vessels. 800×
in the nucleus, one nucleolus and nuclear membrane were clearly observed.

Some of these cells were stained with deep purple black granules and the others were stained just as if painted all over with dark purple black. In the axons, in some of them could be observed the GOMORIphile substance. There were found out closely concentrated GOMORIphile granules surrounding the blood capillaries of postpituitary circumstance as named 'Verdichtungszone' by ROMEIS.

Occasionally, GOMORIphile granules infiltrated into the capillaries or grouped to some masses here and there, and they were observed as so-called HERRING's bodies.

**Fig. 3.** Nucleus supraopticus of the rabbit after the repeated hypoglycaemic shocks. 6 µ. COMORI's staining. GOMORIphile substances are remarkably reduced from the protoplasm of the cell. 4,000×

**Fig. 4.** Posterior pituitary at the time of alloxan shock. 6 µ. GOMORI's staining. GOMORIphile substances are almost disappeared, granules and HERRING's bodies of the posterior lobe are also disappeared. 800×
At the first hypoglycemic shock, the GOMORIphile substances of the nerve cells were generally reduced and the oftener the shocks were repeated, the more appeared the reducting tendency of the GOMORIphile substances. Namely, the cells which were so-called plasmic-stained, painted all over in the case of normal cells, disappeared and their granules were pulverized.

About the nuclei, light stained nuclei as normal ones diminished, and the nuclei were stained a faint purpel blue.

On the other hand, about the post-pituitary, GOMORIphile substances were remarkably reduced, HERRING’s bodies were almost invisible and around the post-pituitary, there were found out especially no groupings of the granules. The oftener the hypoglycemic shocks were repeated, the more appeared the tendency of the GOMORIphile substances reduction and at last, no GOMORIphile granules were found.

b) Stadium of the 7—10 day after the alloxan administration.

GOMORIphile granules of neurosecretions cells and post-pituitary of the rabbit which showed over 200 mg/dl blood sugar level after 7—10 days of alloxan administration, were reduced remarkably on nucleus supraopticus and nucleus paraventricularis, and there were only a few fine granules in the cell body. The nucleolus was clearly visible but somewhat pale compared with normal one.

The degree of granules reduction in the nerve cells was stronger than that of the first hypoglycemic shock, and that degree of reduction was parallel to the degree of hyperglycemy.

At the post-pituitary, the reduction of granules was clearly observed and HERRING’s bodies also decreased and shrank; the so-called ‘Verdichtungszone’ by ROMEIS also almost disappeared.

Fig. 5. Nucleus supraopticus of the rabbit which administrated alloxan before 7 days. (blood sugar level: 324 mg/dl) 6 μ. GOMORI’s staining. Remarkable reduction of the GOMORIphile substances is observed. 4,000×
The degree of these granules reduction of the post-pituitary was recognized to have parallel relation with blood sugar level, but was less than that of shock stadium.

c) Stadium of continued hyperglycemia.

GOMORIphile granules of neurosecretory cells of the rabbit which showed continuously over 200 mg/dl blood sugar level for over one month after alloxan administration were almost more reduced than that of normal one. Especially, the

![Image 1](image1.png)

**Fig. 6.** Posterior pituitary of the rabbit which administrated alloxan before 7 days. (Blood sugar level: 324 mg/dl) 6 u. GOMORI's staining. GOMORIphile substances are reduced, HERRING's bodies are shrunk and so-called 'Verdichtungs zone' is almost disappeared. 800×

![Image 2](image2.png)

**Fig. 7.** Nucleus supraopticus of the same rabbit as in Fig. 7. 6 u. GOMORI's staining. GOMORIphile substances are slightly decreased generally, but observed just as a normal. 4,000×
rabbit which showed continued high blood sugar level for several months only showed a little reduction. At the post-pituitary, granules reduction was observed and shrunken HERRING's bodies and the decreases in their numbers were also observed.

The grouped granules of post-pituitary circumstance were also slightly reduced.

d) Observation upon sulfonylurea administrated rabbits.

There were almost no remarkable changings on the nerve cells of nucleus supraopticus et paraventricularis of the rabbit which were administrated 0.25 g/kg BZ 55 or D 860 daily for 10 days but some of them showed the decreasing tendency of the GOMORIphile granules. Many of nuclei of the cells were stained dark tone compared with normal ones and showed the same stainability as cytoplasm.

And at some of the posterior pituitary, reductions of granules and HERRING’s bodies were observed compared with normal stage.

III. Discussion.

Neurosecretion of hypothalamus and post-pituitary of alloxan diabetic rabbits and sulfonylurea administrated rabbits were investigated by GOMORI's CHP staining method.

At the hypoglycemic shock after alloxan administration, GOMORIphile substances of post-pituitary were remarkably reduced and at length, the granules almost disappeared but with nerve cells of nucleus supraopticus et paraventricularis, the reduction was comparatively slight. GOMORIphile granules in the neurosecretions cells of the rabbit which showed continuous hyperglycemia after alloxan administration for 7—10 days, were remarkably reduced, and the degree of reduction was parallel to that of hyperglycemia. About the posterior pituitary, GOMORIphile granules also reduced but generally not so much as at the first alloxan hypoglycemic
shock.

GOMORIphile granules in neurosecretary cells of the rabbit which showed continued hyperglycemia for over one month had slight reducing inclination and about the postpituitary, also a little reduction was observed.

Throughout these results, the following are conjectured:

When sudden hypoglycemic shock attacked, post-pituitary GOMORIphile substances were turned out at once, but hypoglycemic influence upon the nucleic cells was less strong than that upon post-pituitary, and the oftener the shocks attacked, the stronger became their influences upon the hypothalamic nuclei.

Hyperglycemic stadium continued for 7—10 days, it is supposed that the GOMORIphile substances secretion of nucleic cells and those derived from post-pituitary, were almost balanced, though the latter decreased.

Adaptation was seen for continued hyperglycemia for several months, so the changes of nuclei and post-pituitary were less remarkable than those at the hypoglycemic shock and during shortly continued hyperglycemia.

In most cases, there were to be seen no remarkable changings upon the nucleic cells and the post-pituitary of sulfonylurea administrated rabbit and this internal medecine might have no remarkable influences upon the normal rabbit.

KRATZSCH asserts that the cause of diabetic polyuria is due to the abnormal hormone secretion of post-pituitary, and FUJITA et al. also agree with him.

I suppose that these neurosecretions disorder by experimental diabetes may have some relation with diabetic polyuria, but I can not help doubting whether these disorders are really the cause of polyuria, or on the contrary, are the result of polyuria.

IV. Summary.

1. GOMORIphile substances were periodically observed in the hypothalamo-hypophysial neurosecretary system of alloxan diabetic rabbits and sulfonylurea administrated rabbits.

2. On the alloxan diabetic rabbits, GOMORIphile granules in nucleus supra-opticus et paraventricularis and post-pituitary were generally reduced and significant differences among each periods were recognized.

3. Throughout these results, the changes of neurosecretion were considered to have some relation with experimental diabetic polyuria.

4. About the sulfonylurea administrated rabbits, some of them showed the reduction of GOMORIphile substances in post-pituitary.

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視床下部下垂体の神経分泌に就てGomori氏CHP染色を用いて検索し、次の所見を得た。

1. アロキサン低血糖ショック時におけるGomori好性物質は、視床下部神経分泌核（視束上核、脳室尾核）において一般的にやや減少を示し、ショック発作回数の多くなる程その傾向は一層顕著となる。下垂体後葉では第1回発作時から急激な減少を来し、Herring氏小体及びVerdichtungszoneも殆ど認め難しい。

2. 7-10日間の高血糖期を経過したものでは、神経核の分泌顆粒は著明に減少するものが多く、その程度は第1回低血糖ショック発作時よりも高度であることが多い。後葉においても著明に減少し、Herring氏小体の小形化、Verdichtungszoneの不鮮明等が認められるが、低血糖ショック時の如く甚しくはない。

3. 1-5ケ月間高血糖を持続せるものでは、神経核に於ても、後葉に於てもGomori好性物質はやや減少したものが多くあったが、殆ど正常と異らないものも認められた。

4. プルファニール尿素剤0.25g/kg 10日間経続投与家児に於ては、視床下部神経分泌核及び下垂体後葉のGomori好性物質は殆ど変化を認めなかったが、少数に於て軽に減少を示すものを認めた。

References.