The Blood Sugar Content of the Cold-punctured ("Zwischenhirnstich" of E. Leschke) Rabbit.

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INTRODUCTION.

Cl. Bernard¹⁾ as the first discovered that when mice were under-cooled and the body temperature was not so rapidly fallen, the liver sugar was not only reduced, but disappeared. Since Bernard's discovery, researches concerning the influences of cooling of animal bodies on the metabolism of carbohydrate have been frequently published.

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1) Cl. Bernard, *Leçon de physiologie expérimentale*, Paris 1855, I, 189.

R. Boehm and F. A. Hoffmann¹⁾ observed that when cats, which were not tied down on the holder, were put in the ice bath repeatedly, their body temperature fell and the glycogen was found reduced or absent from their liver and muscles, if the cooling was not too rapid. T. Araki²⁾ could detect the presence of glucose, protein and lactic acid in the urine of the rabbits and dogs whose body temperature descended to 26–24°C., by packing them in snow.

R. Lépine³⁾ produced hyperglycaemia in dogs by means of cold bath. The body temperature fall of these dogs was not remarkable. By the same procedure, H. Freund and F. Marchand⁴⁾ also produced hyperglycaemia in two rabbits whose body temperature fell to 24° and 26°C., but the blood sugar content of one rabbit with the body temperature of 23°C. was normal. They also cooled the abdominal wall of the fastened rabbits by ether. Their body temperature fell two to five degrees in fifteen minutes. They detected hyperglycaemia in four rabbits under the seven. Recently, O. Asakawa⁵⁾ observed also hyperglycaemia in rabbits which were cooled to about 35°C. by means of cold bath. This hyperglycaemia was more remarkable in starving rabbits than in those of good nutrition.

The cold-glycosuria in frogs was studied by E. Pflüger⁶⁾ and specially by M. Loewit.⁷⁾

Besides the above quoted researches in which the body temperature of animals became subnormal by cooling, there are some studies in the influence of the temperature of surrounding atmosphere on the blood sugar content of animals.

The view that the blood sugar content of animals is greater at lower than at higher outer temperature is supported by G. Embden, H. Lüthje and E. Liefmann⁸⁾ (on dogs; the room temperature 2°–32°C.; no note of the body temperature of animals), I. Bang⁹⁾ (on one rabbit; the room temperature 4–5°→20°C.; no note about the body temperature) and H. Freund and F. Marchand⁴⁾ (on rabbits; 5–18°→31–34°C.; the body

1) R. Boehm and F. A. Hoffmann, *Arch. f. exp. Path. u. Pharm.*, **8** (1878), 375.

2) T. Araki, *Ztschr. f. physiol. Chem.*, **16** (1892), 453.

3) R. Lépine, *Le diabète sucré*, Paris 1909, 192.

4) H. Freund and F. Marchand, *Arch. f. exp. Path. u. Pharm.*, **73** (1913), 276.

5) O. Asakawa, *Mitteil. med. Fakult. Univers. Tokyo*, **25** (1921), 550.

6) E. Pflüger, *Arch. f. gesamt. Physiol.*, **118** (1907), 309.

7) M. Loewit, *Arch. f. exp. Path. u. Pharm.*, **60** (1909), 1.

8) G. Embden, H. Lüthje and E. Liefmann, *Beitr. chem. Physiol. u. Path.*, **10** (1907), 275.

9) I. Bang, *Der Blutzucker*, Wiesbaden 1913, 51.

temperature was almost constant. There are some exceptional cases in their experiments.) Contrary to them, B. Kramer and H. W. Coffin¹⁾ observed the increase of the blood sugar content in dogs, first by keeping them longer than twenty four hours in a cold place.

Recently, O. Asakawa²⁾ could not find any variation of the blood sugar content of rabbits by taking them from a hotter room into a colder. The blood sugar content was estimated by the micromethod of I. Bang. The findings of Asakawa are quite confirmed by Mr I. Fujii in this Laboratory. (Fujii's work shall be published later.)

So far as lowering of the temperature of surrounding atmosphere has no effect on the body temperature of animals, it cannot affect the blood sugar content of animals; but if the body temperature fall is somewhat remarkable, the latter increases.

Some explanations of this cold-glycosuria were put forward by E. Pflüger³⁾ and others. But, they need experimental proofs, and look inconclusive.

Powerful reflex effect of the cooling of skin upon the central nervous mechanism or anaemia was considered as the cause of it.^{3) 4)} R. Lépine attributed it to a defensive reaction.

The lowering of the body temperature was attained formerly only by the cooling of the skin of animals by means of an ice bath, snow packing or contact with mercury. The reflex effect of the severe cooling of the skin and the fall of the body temperature, therefore, could not be separated from each other. To analyse the mechanism of the cold-glycosuria, it is very desirous to get the body temperature fall of animals without cooling their skin artificially. And this desire may be easily attained by excluding the central mechanism of the regulation of the body temperature. Various methods were devised to remove this central mechanism. Out of them, I have adopted E. Leschke's "inter-brain-puncture"⁵⁾ by reason of the simplicity of the procedure, and improved it.

The section under the cervical spinal cord undoubtedly causes the fall of the body temperature, but the path from the supposed "sugar center" in the medulla oblongata to its effective organ is also necessarily cut off.⁶⁾

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1) B. Kramer and H. W. Coffin, *Journ. Biol. Chem.*, **25** (1916), 423.

2) O. Asakawa, *Mitteil. med. Fakult. Univers. Tokyo*, **25** (1921), 527.

3) E. Pflüger, *Das Glycogen u. seine Beziehung z. Zuckerkrankh.*, 2 Aufl., Bonn 1905, 528; *Arch. f. gesamt. Physiol.*, **118** (1907), 309.

4) T. Araki, *Ztschr. f. physiol. Chem.*, **15** (1892), 335 and **19** (1894), 422.

5) E. Leschke, *Ztschr. f. exp. Path. u. Ther.*, **14** (1913), 167.

6) R. Boehm and F. A. Hoffmann, *Arch. f. exp. Path. u. Pharm.*, **8** (1878), 422.

The heating of the corpus striatum on rabbits causes the fall of the body temperature, but only to a small extent.¹⁾²⁾³⁾⁴⁾⁵⁾

The removal of the central apparatus for the regulation of the body temperature was attempted first by I. Ott.⁶⁾ Independently of him, R. Isenschmid and L. Krehl⁷⁾ discovered, that after the removal of the end-brain and inter-brain on rabbits their body temperature fell, and they behaved as poikilothermic. Isenschmid⁸⁾ observed further with W. Schnitzler on rabbits, that a certain portion of the tuber cinereum plays an especially important rôle in the central mechanism for the regulation of the body temperature and the conduction to the spinal cord passes through the lower central part of the inter-brain.

By simplifying the process of Isenschmid and Krehl, E. Leschke⁹⁾ rendered rabbits poikilothermic by a puncture between the inter-brain and mid brain. He named this method "Zwischenhirnstich."

Infections, toxins and other pyretic substances could not cause any rise of temperature on the inter-brain punctured rabbits.¹⁰⁾ The warming of the carotid arteries did not alter the frequency of respiration of the same rabbits while the body temperature was raised.¹¹⁾

The central mechanism for the regulation of the body temperature seems, therefore, to be excluded from its effective organs by the "Zwischenhirnstich."

Studies about the carbohydrate metabolism on an animal without the regulation of the body temperature are rare and seem to be not satisfactory.

Leschke could not detect glycosuria on his rabbits. Ko. Naito¹²⁾ estimated the blood sugar content of rabbits in two or four hours after the interbrain-puncture. Hyperglycaemia could not be detected. The maximal temperature fall of his rabbits was about 6°, perhaps owing to the shortness of his observations.

By studying the body temperature and the blood and urine sugar content of inter-brain punctured rabbits systematically till their death, I detected, that hyperglycaemia and glycosuria were induced in them, as soon as the body temperature fell beyond a certain limit. Of this several features shall be presented in the following pages.

- 1) H. G. Barbour, Arch. exp. Path. u. Pharm., **70** (1912), 1.
- 2) M. Hashimoto, Arch. f. exp. Path. u. Pharm., **78** (1915), 394.
- 3) H. G. Barbour and A. L. Prince, Journ. Exp. Pharm. and Therap. **6** (1913), 1.
- 4) L. M. Moore, Amer. Journ. Physiol., **46** (1918), 253.
- 5) A. L. Prince and L. Hahn, Amer. Journ. Physiol., **46** (1918), 412.
- 6) I. Ott, quoted in R. Isenschmid and W. Schnitzler, Arch. f. exp. Path. u. Pharm., **76** (1914), 216.
- 7) R. Isenschmid and L. Krehl, Arch. f. exp. Path. u. Pharm., **70** (1912), 109.
- 8) R. Isenschmid and W. Schnitzler, Arch. f. exp. Path. u. Pharm., **76** (1914), 202.
- 9) E. Leschke, Ztschr. f. exp. Path. u. Ther., **14** (1913), 167.
- 10) J. Citron and E. Leschke, Ztschr. f. exp. Path. u. Ther., **14** (1913), 379.
- 11) Daizo Ogata, Mitteil. med. Fakult. Univers. Kyushu, **4** (1917), 11.
- 12) Ko. Naito (内藤綱一), Tohoku Igaku-Zasshi, **4** (1919), 128. (Jap.)

II. METHODS.

The animal experimented on: Matured male rabbits of good nutrition, which are fed with *tofukara* over two weeks in our Laboratory (see I. Fujii, this Journal this volume p. 172).

Blood samples: From the posterior branch of the auricular vein. To draw blood samples with facility and without causing any pain on the animal, the cervical sympathetic and the great auricular nerve on that side are severed without narcotics three days before an experiment.

The collection of the urine: From time to time animals were fastened on the holder and the urine was collected by catheterization. (Punctured rabbits can not be catheterized by free hand, owing to the increased reflex irritability.)

The measurement of the body temperature: The calibrated thermometer was inserted 5-7 cms. into the rectum of animals. Wait till the tip of the mercury column becomes stationary and then read off.

The estimation of the blood sugar content: By the micromethod (1913) of I. Bang.¹⁾ (Two blood samples were taken every time.)

The estimation of the liver glycogen content: By the method of H. Bierry and Z. Gruzewska.²⁾

The estimation of the sugar content in the urine: By Bertrand.³⁾ If the urine contained proteins, they were removed by Michaelis-Rona⁴⁾ before the estimation of the sugar content.

The splanchnectomy: By O. Schultze⁵⁾, without narcotics. Experiments were not made until eight days after splanchnectomy.

The cold-puncture (der Kältestich): Mainly by E. Leschke.⁶⁾ The breadth of the puncture or section between the inter-brain and mid-brain was 1-2 mms. on each side from the medial line in the experiments of Leschke, Leschke and Citron, and Ko. Naito. I have widened the breadth of the puncture to 2-3 mms.

The result differs very remarkably from those of Leschke and others.

E. Leschke named this operation "Zwischenhirnstich." But,

1) I. Bang. *Bioch. Ztschr.*, **49** (1913), 19 and **57** (1913), 200.

2) H. Bierry and Z. Gruzewska, *C. R. de l'Acad. des Sciences*, **156** (1913), 1491.

3) G. Bertrand, in A. Morel, *Précis de technique chimique*, Paris 1909, 337.

4) P. Rona and L. Michaelis, *Bioch. Ztschr.*, **7** (1908), 329.

5) O. Schultze, *Arch. f. exp. Path. u. Pharm.*, **43** (1900), 193.

6) E. Leschke, *Ztschr. f. exp. Path. u. Ther.*, **14** (1913), 167.

in fact, the puncture is not made in the inter-brain, as in the "fore-brain puncture" of Ed. Aronsohn and J. Sachs, but between it and the mid-brain. So, this naming may cause misunderstanding. Therefore, it may be more convenient to name this procedure as the "cold-puncture" (Kältestich) from its effect on the body temperature, in analogy to the "heat-puncture" (Wärmestich).

Daizo Ogata translated "Zwischenhirnstich" as the "mid-brainpuncture." "Zwischenhirn" may be translated sometimes as the midbrain, but strictly it must be the inter- or between-brain.

After receiving the cold-puncture, usually animals lie down on one side quietly, the tonus of the skeletal muscles being diminished; while they not seldom crouch in one place. On animals whose body temperature falls remarkably, owing to the success of the puncture, the tonus of certain muscle-groups increases greatly in 2 to 3 hours after the puncture, whether their behavior belongs to one or the other type of the two. So, the neck and head rigidly retract and the tail is stiff and also retracted. The elbow and knee joints are stiffly extended. Such a state is nothing else than the "decerebrated rigidity" of C. S. Sherrington¹⁾ ("acerebrated tonus" of M. Loewenthal and V. Horsley²⁾). The reflex action can be obtained with great ease on such animals. So, any slight mechanical stimulus of the skin causes rhythmic movements of progression. The punctured rabbits, which exhibited spontaneously rotary or progressive movements vigorously, were seldom seen. They were not used to the experiment.

The cold-puncture causes the animals great pain.

A great fall of the body temperature could not be attained by the earlier experimentalists. So in Leschke's experiments,³⁾ the rabbits' body temperature usually descended only to 34–36°C. on the next day after the puncture. The body temperature of 30°C. was attained only in two cases out of the nine rabbits and just before the death. In the experiments of Citron and Leschke,⁴⁾ only one rabbit's body temperature was under 30°C. in case of trypanosoma infection, judging from their curves. R. Isenschmid⁵⁾ could by the method of Leschke, remove the mechanism for the regulation of the body temperature on only one rabbit. Daizo Ogata⁶⁾ described two punctured rabbits. The body temperature of the one was 29.5°C. (the room temperature 16.5°C.) on the next day. It was 33.9°C. (the room temperature 20.3°C.) on the other.

1) C. S. Sherrington, Proc. Roy. Soc. London, **60** (1897), 414 and Journ. of Physiol., **22** (1898), 319, and The integrative action of the nervous system, London 1911, 299.

2) M. Loewenthal and V. Horsley, Proc. Roy. Soc. London, **61** (1897), 20.

3) E. Leschke, Ztschr. f. exp. Path. u. Ther., **14** (1913), 170.

4) J. Citron and E. Leschke, Ztschr. f. exp. Path. u. Ther., **14** (1913), 379.

5) R. Isenschmid, Arch. f. exp. Path. u. Pharm., **75** (1914), 15.

6) Dai. Ogata, Mitteil. med. Fakult. Univers. Kyushu, **4** (1917), 17.

So, the only criticism about the inter-brain puncture of Leschke (Isenschmid,¹⁾ M. Ishihara,²⁾ D. Ogata³⁾) is that the mechanism for the regulation of the body temperature could not be entirely removed, but only somewhat damaged. It seems to be correct, judging from their experiments, or further, concerning the proper method of Leschke himself.

In the results of my process of the cold-puncture, which differs only concerning the breath of the puncture or section, there is a very great difference compared with that of Leschke. While the rabbits of Leschke, and Citron and Leschke died in 4-6 days after the puncture, a majority of the rabbits in my experiments died on the next day after the puncture and seldom one or two days later [except on very hot days as in August (about 28-30°C. of room temperature)]. And the velocity of the fall of the body temperature was very great. In only five hours, it descended to 33°C. and finally always under 30°C. In winter it fell to 14-15°C. in some rabbits. So, in the late stadium, the body temperature was a little higher than the room temperature and varied parallel with the latter.

The velocity, with which the body temperature fell in some hours after the puncture, resembles that of the inter-brain removal of Isenschmid.⁴⁾ His rabbits' body temperature fell in some hours to 30°C. or further in the room of 18°C.

The velocity and maximum of the body temperature fall depend upon the breath of the puncture mainly. But the season has also some influence upon them. While in winter (in November, December and January) it fell to 26°C. in ten hours after the puncture, in summer (in May and June) it seldom fell to 30°C. in the same time interval. So, in winter animals died the next morning after the puncture and in summer the next night usually.

The cold-punctured rabbits did not eat voluntarily, as did those with the inter-brain removal of Isenschmid.⁴⁾ In my experiments, no food was given to the animals artificially.

On some rabbits whose body temperature fall was not quick, but ceased in a certain degree or rose again a little (though it never reached the initial value), it was discovered by autopsy that the section was insufficient and often especially the section on one side of the medial line was missing.

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1) R. Isenschmid, *Arch. f. exp. Path. u. Pharm.*, **75** (1914), 1.

2) Makoto Ishihara (石原誠), *Nisshin-Igaku*, **4** (1915), 1095. (Jap.)

3) Daizo Ogata, *Mitteil. med. Fakult. Univers. Kyushu*, **4** (1917), 17.

4) R. Isenschmid, *Arch. f. exp. Path. u. Pharm.*, **75** (1914), 18.

So, the cold-puncture, essentially improved, can remove the central mechanism for the regulation of the body temperature just as the removal of the inter-brain of Isenschmid and Krehl; though it is a very simple procedure in comparison with the latter. To get a constant results of the puncture some practices are necessary.

III. THE COLD-PUNCTURE ON NORMAL RABBITS.

Hyperglycaemia may be induced on rabbits, cats, etc. by tying down on the holder, stimulation of sensory nerves, emotional disturbances, narcosis and other causes. Recently, I. Fujii reported that "Fesselungsdiabetes" is a quite constant occurrence in rabbits, even in rabbits fastend a very short time. The above process, the severing of the paths from the central mechanism for the temperature regulation to the periphery between the inter- and mid-brain, itself may also certainly cause hyperglycaemia. Therefore, the degree and course of this operation hyperglycaemia must be first known, as a control to the principal experiment.

On the day previous to the principal experiment, the control was performed except in some cases as follows: The first blood sample was drawn from the ear vein of the rabbits which were sitting freely in a large pan and the second sample in the same manner about one hour later. The rabbits were then tied down on the holder with the face down, and the urine was collected by catheterization. The skull-bone was perforated, venous sinus ligated in two places and then the wound closed by means of small artery forceps. The rabbits were then set free. No narcotic was used.

The third blood sample was drawn about half an hour after the second; then once in every hour blood samples were collected. Afterwards the body temperature was measured every time after drawing blood samples. The urine was collected only once some hours after the operation. Rabbits were tied down for the catheterization.

The control operation induced a temporary increase of the blood sugar content. The urine sugar content did not increase at all or scarcely any. In nearly all cases, the blood sugar drawn immediately after the operation was maximal and exceeded the initial value by 0.01-0.07%. Generally the excess is greater in winter than in summer. The blood sugar decreased then gradually and reached again its initial value in about 1, 2-6 hours. In some cases it descended further a little, but

it did not exceed the physiological limit of the blood sugar content. The body temperature fall usually occurred temporarily immediately after the operation, but it did not exceed one degree. Finally it sometimes exceeded the initial value a little, but still within the physiological limit. Proteins in the urine were discovered in only one case.

1. The blood and urine sugar content in normal rabbits resultant from the cold-puncture.

The food (*Tofukara*) was given to animals after the control experiment. They ate as usual.

On the next morning, the initial blood sugar was drawn from the animal which was sitting quietly in the large pan. The rabbit was then tied down on the holder, the urine collected, the body temperature measured and opening the wound on the head, the cold-puncture was performed. Then the wound was sewed and the animal was set free. The collection of blood samples and the urine and the measurement of the body temperature was performed, just as in the control experiment. The cold-punctured rabbits did not take food. No food was given them. On the next days after the puncture, the collection of blood samples and measurement of the body temperature were made every three, four or five hours. The urine was collected one or two times a day. For this the animals were always tied down. Spontaneous urination was also examined.

The cold-puncture caused a rapid and extreme fall of the body temperature as described already in the previous chapter. The body temperature descended under 33°C. in 4–5 hours after the puncture and in the majority it reached 30°C. in 8–9 hours after the puncture. At the latest, the next morning the body temperature was found to be under 30°C. When it reached to 22–33°C., it varied with the room temperature. Animals died with a body temperature of about 20°C., seldom of 14–15°C.

It is generally assumed that when rabbits are cooled to a body temperature of 19°C., they die; while a body temperature of 12.5°C. has been experienced in a monkey.¹⁾ The tetani, which R. Winternitz²⁾ observed on cooled rabbits with the body temperature of 22–19°C., were never seen on the cold-punctured rabbits. The body temperature of 14.3° and 13.7°C. was observed on the cold-punctured rabbits with bilateral splanchnectomy. The former lived 2.5 hours after the body temperature descended under 15°C.

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1) R. Tigerstedt, Winterstein's Handb. d. vergl. Physiol. III, 2 (1910–1914), 87.

2) R. Winternitz, Arch f. exp. Path. u. Pharm., 33 (1905), 291.

TABLE
The blood sugar content resultant from

No.	Control experiment										
	Date	Body weight	Initial blood sugar	Maxi- mal blood sugar after operation	Hours to the max. blood sugar	Aug- menta- tion of blood sugar	Date	Body weight	Initial		Maxi- mal blood sugar
									blood sugar	body temp.	
(kgms)	(%)	(%)	(%)	(%)	(kgms)	(%)	(°C.)	(%)			
1							27. VI. '19	1.37	0.09	38.8	0.15
2							22. IX. '19	1.80	0.14	38.0	0.23
3	9. X. 19'	1.85	0.12	0.19	$\frac{1}{6}$	0.07	10. X. '19	1.89	0.11	39.3	0.18
4	27. X. '19	2.12	0.12	0.18	$\frac{1}{4}$	0.06	28. X. '19	2.20	0.10	39.2	0.18
5	27. X. '19	2.02	0.14	0.19	$\frac{1}{6}$	0.05	28. X. '19	2.13	0.12	38.9	0.17
6	6. XI. '19	1.69	0.11	0.16	$\frac{1}{12}$	0.05	7. XI. '19	1.63	0.11	39.2	0.14
7	16. I. '20	1.65	0.13	0.15	$\frac{1}{4}$	0.02	17. I. '20	1.61	0.12	38.8	0.17
8	17. V. '20	1.78	0.13	0.15	$\frac{1}{4}$	0.02	18. V. '20	1.87	0.13	38.5	0.16
9	21. V. '20	1.83	0.12	0.14	$\frac{1}{6}$	0.02	22. V. '20	1.84	0.12	38.3	0.16

Immediately after the puncture, the blood sugar content increased and gradually returned to the initial value in 1-4 hours. The degree and course of this hyperglycaemia agrees nearly with that of each control experiment. Therefore this is the "operation" hyperglycaemia. The urine sugar content did not increase.

In the majority of cases, the course of the blood sugar content after the elapse of the "operation" hyperglycaemia was as the above examples. After elapse of the "operation" hyperglycaemia, the blood sugar content held its initial value till the body temperature descended further and reached to about 30°C. As soon as it became less than 30°C. and usually 28-29°C., the blood sugar content began to increase suddenly. The time interval from the cold puncture till the beginning of this hyperglycaemia was about ten hours or more. It depended on the velocity of the fall of the body temperature. The hyperglycaemia

I.

the cold-puncture on the normal rabbit.

Principal experiment

Hyperglycaemia after puncture			Minimal blood sugar after puncture			Blood sugar at the body temperature under 30°C.				Hours after puncture till death	Blood sugar before the rabbit is died	Body temp.
Body temp. at the max. blood sugar	Hours to it after puncture	Augmentation of blood sugar	Blood sugar	Body temp. at the min. blood sugar	Hours to it after punct.	Hours after puncture till the beginning of hyperglycaemia	Body temp. at	Maximal blood sugar	Duration of hyperglycaemia			
(°C.)		(%)	(%)	(°C.)			(°C.)	(%)	(hours)		(%)	(°C.)
34.5	2 $\frac{1}{3}$	0.06	0.09	30.4	7 $\frac{1}{2}$					>33	0.08	27.1
36.9	$\frac{1}{2}$	0.09	0.10	34.7	26					>56	0.14	28.9
37.7	$\frac{1}{8}$	0.07	0.10	30.5	7 $\frac{1}{4}$	9 $\frac{1}{4}$ < 23 $\frac{1}{4}$	25.1	0.28	>9			
38.6	$\frac{1}{6}$	0.08	0.11	28.8	7 $\frac{1}{6}$	9 $\frac{1}{6}$	28.4	0.25	41	50 $\frac{1}{6}$	0.19	20.0
37.8	$\frac{1}{12}$	0.05	0.10	32.4	9	11 < 23 $\frac{1}{4}$	28.1	0.25	>33			
38.0	$\frac{1}{12}$	0.03	0.11	31.5	3	9 $\frac{1}{6}$ < 22 $\frac{3}{4}$		0.30	>9			
36.2	$\frac{1}{3}$	0.05	0.12	30.0	3 $\frac{1}{3}$	6 $\frac{2}{3}$	28.0	0.21	>8			
36.4	1	0.03	0.12	30.0	11	13 $\frac{1}{6}$ < 22 $\frac{3}{4}$		0.22	>12	48	0.22	18.5
37.1	$\frac{1}{3}$	0.04	0.13	31.4	8	10	31.2	0.34	42			

increased with the lapse of time and the fall of the body temperature until the death of animals. So, it extended two days. But, on the third day hyperglycaemia inclined to decrease with the lapse of time, notwithstanding the body temperature further fell or ascended a little parallel to the room temperature. In one case, hyperglycaemia decreased somewhat on the next day after the puncture, while the body temperature further fell.

The maximum blood sugar content was on the next day after the puncture and its value was 0.2–0.3%, seldom over 0.3%.

This hyperglycaemia was accompanied necessarily by glycosuria and mostly by albuminuria, contrary to the control experiment. T. Araki¹⁾ discovered proteins besides glucose and lactic acid in the urine of the

1) T. Araki, Ztschr. f. physiol. Chem., 16 (1892), 453.

EXPERIMENT III.

Date	Body weight (kgrms)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (C°.)	Urine				
						Quantity (c.c.)	Reaction	Specific gravity	Protein	Sugar (%)
28. IX. 1919	1.79	Section of r. cervical sympathetic and r. auricular nerve.								
9. X. "	1.85	11.00 A.M.	0.12	38.4	19.2					
		1.00 P.M.	0.12	38.5	20.2					
		1.08	Fettered.							
		1.10				35	alkaline	1015	—	0.068
		1.15-1.25	Control operation.							
		1.27	Set free.							
		1.30	0.19	38.4	21.2					
		2.30	0.16	38.6	21.3					
		3.30	0.13	38.8	22.2					
		4.30	0.12	39.0	22.2					
		5.30	0.10	39.1	22.8					
		6.30	0.10	39.0	22.2					
		6.40				40	acid	1015	—	0.072
10. X. "	1.89	8.00 A.M.	0.11	39.3	17.8					
		9.08	Fettered.							
		9.10				70	alkaline	1018	±	0.012
		9.12-9.20	Punctured (9.18), afterward fell down to the right side.							
		9.22	Set free.							
		9.30	0.18	37.7	19.2					
		10.30	0.15	35.1	19.9					
		11.30	0.14	33.8	20.4					
		12.30 P.M.	0.13	32.8	20.8					
		12.40				7	alkaline		±	0.048
		1.30	0.12	31.9	21.0					
		2.30	0.11	31.3	21.4					
		3.30	0.10	30.9	21.4					
		4.30	0.10	30.5	22.0					
		5.30	0.11	30.5	22.2					
		6.30	0.12	30.4	22.3					
		From 5 o'clock afternoon convulsion on the paws.								
11. X. "	1.75	8.30 A.M.	0.19	25.1	16.4					
		8.40	Urinates spontaneously.			5	acid		trace	1.69
		11.30	0.21	23.8	18.7					
		2.30 P.M.	0.25	23.8	21.5					
		5.30	0.28	24.5	22.2					
		5.40				2	acid			2.59
		Died in the night.								

EXPERIMENT IV.

Date	Body weight (kgrms)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)	Urine				
						Quantity (c.c.)	Reaction	Specific gravity	Protein	Sugar (%)
7. X. 1919	2.10	Section of r. cervical sympathetic and r. auricular nerve.								
27. X. „	2.12	10.00 A.M.	0.11	38.7	15.2					
		11.00	0.11	38.9	16.2					
		11.08	Fettered.							
		11.14-11.17	Control operation.							
		11.18	Set free.							
		11.30	0.18	38.8	16.3					
		12.30 P.M.	0.16	38.8	17.8					
		1.30	0.13	38.8	18.3					
		2.00				12	acid		—	
		2.30	0.13	39.2	19.1					
		3.20	0.12	39.3	20.2					
		4.30	0.12	39.5	20.8					
		5.30	0.11	39.3	21.2					
		7.30	0.12	39.3	21.5					
		Appetite good, from 2 o'clock no urine is purged until the next morning.								
28. X. „	2.20	8.30 A.M.	0.10	39.2	16.2					
		8.43	Fettered.							
		8.45				95	alkaline	1027	—	0.026
		8.46-8.55	Punctured (8.53), afterwards fell down to the left side.							
		8.56	Set free.							
		9.00	0.18	38.6	17.1					
		10.00	0.14	36.4	17.8					
		11.00	0.11	33.2	18.8					
		12.00	0.12	32.2	20.2					
		12.15 P.M.				5	alkaline		—	0.026
		1.00	0.13	31.0	20.5					
		2.00	0.12	30.2	20.6					
		3.00	0.12	29.3	20.6					
		4.00	0.11	28.8	20.7					
		4.30	Sit down straight.							
6.00	0.19	28.4	21.6							
8.00	0.20	28.8	22.6							
29. X. „	2.03	8.00 A.M.	0.25	25.6	17.0					
		11.00	0.22	25.1	19.5					
		2.00 P.M.	0.23	24.6	20.0					
		2.20								
		5.00	0.21	24.1	20.1					
On this day, lay down to the left side always.										
30. X. „	1.96	8.00 A.M.	0.20	19.5	16.5					
		11.00	0.19	20.0	19.8					
		12.00				10	acid		+	2.19
		12.05 P.M.	Died at the time of catheterization.							

EXPERIMENT VII.

Date	Body weight (kgms)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C)	Urine						
						Quantity (c.c.)	Reaction	Specific gravity	Protein	Sugar (%)		
12. I. 1920.	1.62	Section of r. cervical sympathetic and r. auricular nerve.										
16. I. ,	1.65	10.30 A.	0.13	38.4	13.1							
		11.30	0.13	38.3	14.0							
		11.40	Fettered.									
		11.42										
		11.45-11.50	Control operation.									
		11.52	Set free.									
		12.00	0.15	37.8	15.2							
		1.00 P.M.	0.13	38.9	16.3							
		2.00	0.14	39.0	19.3							
		3.00	0.12	39.2	17.2							
		4.00	0.13	39.1	18.3							
		5.00	0.11	39.1	17.8							
		5.10				20	acid		-	0.036		
		7.00	0.11	39.1	16.2							
		17. I. ,	1.61	8.50 A.M.	0.12	38.8	10.0					
				9.00	Fettered.							
9.02						30	acid	1013	-	0.024		
9.05-9.14	Punctured (9.10), bled a large quantity, afterwards sit down straight.											
9.15	Set free.											
9.30	0.17			36.2	11.8							
10.30	0.15			32.8	14.0							
11.30	0.12			30.9	15.8							
12.30 P.M.	0.12			30.0	16.9							
1.30	0.13			29.2	17.8							
3.30	0.16			28.0	19.2							
3.50	Clonic convulsion.											
5.30	0.15			27.0	18.8							
6.00						13	acid		-	0.108		
7.30	0.21	26.2	17.7									
9.30	0.17	23.8	16.2									
11.30	0.20	21.9	15.0									
On this day crouch always, died in the night.												

rabbits and dogs with the body temperature of 26–24°C., by packing them with snow. M. Loewit¹⁾ always induced albuminuria in frogs by cooling, while glycosuria was not a constant phenomenon.

The course and length of the cold-puncture hyperglycaemia differ from that of the piqûre of Cl. Bernard. In the latter, the blood sugar content begins to increase immediately after the puncture, reaches its maximum in a short time and continues only several hours.^{2,3)4)5)} By the piqûre, the body temperature falls also, but to a small extent, i.e. it is far from 30°C.⁶⁾

This comparison may be not quite justified, because the cold-puncture hyperglycaemia occurs on the next day after the puncture i.e. on the second day of fasting, whereas the piqûre hyperglycaemia of E. Neubauer and others was studied on rabbit of good nutrition.

One rabbit, in which cold-puncture was not successful i.e. its body temperature was 33–34°C. even on the third day and the blood sugar content was quite normal, except for the operation hyperglycaemia, was utilized for the purpose of this comparison. The piqûre of Cl. Bernard was performed on the fourth day of the cold-puncture. The result was as follows :

EXPERIMENT X.

Date	Body weight (kgms)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)	Urine					
						Quantity (c.c.)	Reaction	Specific gravity	Protein	Sugar (%)	
2. VI. 1920	1.99	Section of r. cervical sympathetic and r. auricular nerve.									
13. VI. "	2.00	9.10 A.M.	0.12	38.1	17.8						
		9.35	Fettered.								
		9.36				25	neutral	1025	—	0.024	
		9.40–9.48	Punctured (9.45), bled a little, afterwards lay down to the right side.								
		9.50	Set free.								
		10.00	0.16	37.2	18.3						
		11.00	0.16	35.2	18.8						
		12.00	0.14	34.5	19.0						

1) M. Loewit, Arch. f. exp. Path. u. Pharm., **60** (1909), 1.

2) E. Neubauer, Bioch. Ztschr., **43** (1912), 352.

3) I. Bang, Der Blutzucker, Wiesbaden 1913, 100.

4) Th. Stenström, Bioch. Zeitschr., **58** (1914), 476.

5) Yoshi. Kuno, Mitteil. med. Fakult. Univers. Tokyo, **22** (1919), 172.

6) I. Fujii, this Journal, **1** (19.0), 38.

Date	Body weight (kgms)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)	Urine				
						Quantity (c.c.)	Reaction	Specific gravity	Protein	Sugar (%)
13. VI. 1920		1.00 P.M.	0.13	34.4	18.8					
		3.00	0.13	33.8	19.9					
		5.00	0.12	34.2	20.2					
		7.00	0.12	34.0	20.2					
14. VI. "	1.79	8.30 A.M.	0.11	32.6	17.5					
		8.30	Urine spontaneously.			40	acid	1025	—	0.024
		12.00	0.11	32.7	20.2					
		3.00 P.M.	0.12	32.9	21.0					
15. VI. "	1.69	7.00	0.12	33.4	22.0					
		8.30 A.M.	0.12	33.4	17.2					
		8.40				36	acid	1030	—	0.024
		12.00	0.11	33.7	21.0					
16. VI. "	1.56	3.30 P.M.	0.11	34.1	23.0					
		7.00	0.12	34.1	23.8					
		8.30 A.M.	0.12	34.4	20.1					
		9.20	Fettered.							
17. VI. "	1.52	9.22-9.32	Piqûre of Cl. Bernard (9.30).							
		9.35	Set free.							
		11.20	0.24	34.1	22.2					
		12.20 P.M.	0.28	33.3	22.5					
		2.20	0.29	33.1	23.5					
		8.10	0.17	33.4	23.8					
		7.40 A.M.	0.15	33.0	20.6					
17. VI. "	1.52	11.25				75	alkaline	1050	—	0.650
		12.10 P.M.	0.18	33.2	24.0					
		7.10	0.18	34.2	26.2					

So, on the rabbit of three days' fasting, the piqûre hyperglycaemia was marked. Its course looks quite different than that of the cold-puncture, whereas it is quite identical with that of the piqûre hyperglycaemia on animals of good nutrition. The body temperature of E. Leschke's inter-brain punctured rabbits, in which no glycosuria could be detected, was not under 33-34°C. Ko. Naito found normal blood sugar content in two or four hours after the inter-brain puncture. The body temperature fall did not exceed 34°C. In my experiments, in the same time interval after the puncture as Ko. Naito the body temperature did not yet reach 30°C. and the cold-puncture hyperglycaemia was never detected.

Some exceptional cases were observed, i.e. on some rabbits, whose body temperature fell under 27°C., no hyperglycaemia could be detected until the death of the animals, as follows :

EXPERIMENT I.

Date	Body weight (kgms)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)	Urine				
						Quantity (c.c.)	Reaction	Specific gravity	Protein	Sugar (%)
25. VI. 1919	1.34	Section of r. cervical sympathetic and r. auricular nerve.								
27. VI. "	1.38	9.20 A.M.	0.09	38.8	19.8					
		10.00	0.09	38.8	20.4					
		10.15	Fettered.							
		10.16-10.27	Punctured (10.25), bled a little.							
		10.28	Set free.							
		11.00	0.10	37.5	20.4					
		12.00	0.11	35.2	20.9					
		1.10 P.M.	0.15	34.5	21.3					
		1.50				19	alkaline	1032	—	0.02
		2.00	0.12	34.5	21.8					
		4.00	0.11	34.5	21.9					
		6.00	0.09	34.2	21.2					
		28. VI. "	1.29	8.20 A.M.	0.02	30.4	18.9			
10.30						53	acid	1028	—	0.120
1.50 P.M.	0.11			29.2	22.5					
6.00	0.08			27.1	22.3					
Died in the night.										

2. The glycogen content in the liver of the rabbit, whose body temperature descends under about thirty degrees.

The piqûre of Cl. Bernard does not yield positive results on fasting animals; so it is believed generally that its success depends upon the nutritive condition of animals. Of the adrenaline hyperglycaemia, the same opinion prevails.

So then, if any process is to be recognized as not inducing hyperglycaemia, it must be first verified, that the liver glycogen content of the experimented animals was not abnormally small.

From the fact that the piqûre could not succeed on the rabbits with bilateral adrenalectomy, A. Mayer¹⁾ and R. H. Kahn²⁾ concluded that the piqûre induces hyper-

1) A. Mayer, C. R. de la Société de Biologie, **58** (1906), 1123.

2) R. H. Kahn, Arch. f. gesamt. Physiol., **128** (1909), 302.

glycaemia through adrenals first. But, A. Porges¹⁾ and O. Schwarz²⁾ did not agree with them. The livers of the dogs (Porges) and rats (Schwarz) with bilateral adrenalectomy were free from glycogen reserve.

G. N. Stewart and J. M. Rogoff³⁾, who induced hyperglycaemia on rabbits with bilateral adrenalectomy by the ether narcosis, dyspnoea and piqûre, concluded that the earlier experiments with negative results may be possibly due to deficiency of the liver glycogen reserve.

On the other hand, the reports that the hyperglycaemia by adrenaline, diphtheria toxin and stimulation of the afferent vagus nerve⁴⁾ on fasting rabbits is as strong as on rabbits of good nutrition, are not now infrequent. Quite recently, O. Asakawa⁵⁾ observed that the hyperglycaemia by diphtheria toxin, typhus toxin, adrenaline and cooling in fasting (3-16 days long) rabbits is of the same degree of that in rabbits of normal nutrition.

I have also observed a marked piqûre-hyperglycaemia on a rabbit of three days' fasting (this essay, p. 417-418).

The fasting diminishes the tolerance for glucose in dogs⁶⁾ (? ⁷⁾) and human beings⁸⁾, but not in cats and rabbits.⁹⁾

The diminution of the liver glycogen content in fasting is an unquestionable fact. The diminution seems to be rapid in the first days of hunger and later very gradual. According to the not yet published experiment of Mr I. Fujii in this Laboratory by the method of Bierry and Gruzewska, the liver glycogen content of the rabbit of one day's hunger is about one third of the control rabbits (9 rabbit: The average content 1.278%, 0.566 grm.), that of two days' hunger is about one fourth of the control (8 rabbits: The average content 0.862%, 0.325 grm.). The liver glycogen content (Pflüger's method) of the rabbit of Rolly¹⁰⁾ to which strychnine was given on the third day of starvation, was 0.112% on the twentieth day of hunger. S. Kuriyama¹¹⁾ estimated (Pflüger's method) the liver glycogen content of

1) A. Porges, Wien. klin. Wochenschr., 1908, 1798.

2) O. Schwarz, Arch. f. gesamt. Physiol., **143** (1910), 259.

3) G. N. Stewart and J. M. Rogoff, Amer. Journ. Physiol., **46** (1918), 90 and **51** (1920), 366.

4) I. Bang, M. Ljungdahl and V. Böhm, Beitr. z. chem. Physiol. u. Path., **10** (1907), 1.

5) O. Asakawa, Mitteil. med. Fakult. Univers. Tokyo, **25** (1921), 539.

6) F. Hofmeister, Arch. f. exp. Path. u. Pharm., **26** (1889-90), 355.

7) M. Doyon & E. Dufourt, Journ. de Physiol. et de Path. générale, **3** (1901), 704.

8) G. Hoppe-Seyler, Münch. med. Wochenschr., 1900, 531.

9) F. M. Allen, Studies concern. glycosuria and diabetes, Boston 1913, 43, 192 & 580.

10) Fr Rolly, Deutsch. Arch. f. klin. Med., **83** (1905), 107.

11) S. Kuriyama, Journ. Biol. Chem., **34** (1918), 281.

rabbits of 8–11 days' fasting at 0.28–1.05% (average of five rabbits 0.55%).

Recently A. Imamura and G. Kira¹⁾ reported that the liver glycogen content of rabbits (Pflüger's method) remained at 0.551% (0.2686 grm.), even on the tenth day of hunger, as long as the blood sugar content remained normal. But, when animals were dying of starvation of 7–12 days and their blood sugar content decreased, the liver glycogen content was reduced to about 0.2% or less.

That the liver of rabbits of 2 days' fasting (Luchsinger²⁾) or 2–8 days' fasting (I Bang and his co-workers³⁾) did not contain any trace of glycogen, should be looked upon as possibly an error. The liver of a rabbit of 3 days' fasting of the latter contained only 0.04 grm. glycogen. E. Pflüger⁴⁾ found glycogen of 1.224% in the liver of a dog of 73 days' fasting.

Imamura and Kira¹⁾ observed further, that by subcutaneous injection of adrenaline the blood sugar increased in rabbits of a long fast so long as the blood sugar content remained normal, but it did not occur when the blood sugar content became subnormal, i.e. when the liver glycogen content was reduced to 0.2% or less.

Knowing the report of Imamura and Kira, it may be useful to look into the early communications. The blood sugar of two fasting rabbits of I. Bang⁵⁾ (8 days? p. 91) was 0.12% and 0.09%, before the adrenaline injection. That of three rabbits of K. Sakaguchi, I. Hayashi and S. Yezima⁶⁾ (6–9 days' fasting) was 0.103–0.123% before the injection of diphtheria toxin. So, they were within normal limits and had, therefore, capacity to be hyperglycaemic. In the experiments of K. Sakaguchi,⁷⁾ there were two fasting rabbits whose initial blood sugar was low (0.070% and 0.082%), but hyperglycaemia was produced on them by the injection of peptons and their digestive products. They seem to not quite harmonize with Imamura and Kira's examples (p. 687–688). But it may be assumed that they are also within the physiological limit. On a rabbit, whose initial blood sugar content was 0.032% on the seventh day of fasting peptone did not cause any increase of blood sugar. The animal died soon after. About the same is to be detected in the experiments of Asakawa.

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1) A. Imamura and G. Kira (今村明光, 雲英元孝), *Nippon-Naikwagakwai-Zasshi*, **7** (1920), 676. (Jap.)

2) Luchsinger, quoted in Seegen, *Die Zuckerbildung im Tierkörper*, Berlin 1890, 200.

3) I. Bang, M. Ljundahl and V. Bohm. *Beitr. chem. Physiol. u. Path.*, **10** (1907), 1.

4) E. Pflüger, *Arch. f. gesamt. Physiol.*, **119** (1907), 120.

5) I. Bang, *Der Blutzucker*, Wiesbaden 1913, 89.

6) K. Sakaguchi, I. Hayashi and S. Yezima, *Mitteil. med. Fakult. Univers. Tokyo*, **20** (1918), 78.

7) K. Sakaguchi, *Mitteil. med. Fakult. Univers. Tokyo*, **20** (1918), 485.

Thus, the view of Imamura and Kira can explain some results of early researches. But, as a whole, the relation between the severity and duration of hyperglycaemia and the glycogen content of the liver seems not yet to be settled, but to require further study.

It was detected by I. Fujii¹⁾, that the "Fesselungs"-hyperglycaemia is stronger in winter and spring than in the other seasons. This is nearly parallel to the glycogen content of biceps femoris muscle in dogs, which was estimated by Maignon. The glycogen content of rabbit's liver in winter and summer was determined by numerous experimentalists, but there is no complete investigation of it through one year. The results of Mr I. Fujii, who is engaged with this question in this Laboratory, seem to agree nearly with that of dogs' muscle glycogen content. So the severity of "Fesselungs"-hyperglycaemia depends to some extent on the glycogen content of the liver.

I have also the following experiment: Ammonium chloride solution could induce no hyperglycaemia on a cat, whose nutritive state (judging by her body weight) was not yet recovered after the section of the posterior roots which innervate the hind limb of one side, by hypodermic injection in that hind limb, whereas a marked hyperglycaemia as on normal cats was produced on another similar cat, but of good nutrition, by quite the same process.

The conclusion of the foregoing paragraph is that when the body temperature of the cold-punctured rabbits became less than about 30°C., the blood sugar began to increase suddenly, but infrequently the blood sugar did not increase at all.

The result of the above quoted literature and the fact that the cold-puncture hyperglycaemia is of central origin, as shown in the next chapter, would justify the question as to whether the exceptions may not be due to deficiency of the liver glycogen reserve.

In this way, the cold-puncture was performed on normal rabbits. The blood sugar and body temperature were estimated as usual. At a certain moment, when the body temperature was below about 28°C., the animals were killed with a blow on the neck and the liver was immediately taken out. Its glycogen was then estimated.

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1) I. Fujii, this Journal, 2 (1921), 9.

2) Maignon, quoted in R. Lépine, *Le diabète sucré*, Paris 1909, 114.

As a control, the liver glycogen content of normal (not fasting) rabbits at 9 o'clock in the morning was determined as follows:

TABLE II.

The liver glycogen content of normal rabbit.

No.	Date	Body weight (kgrms.)	Blood sugar (%)	Weight of liver (grms.)	Glycogen content of liver	
					(mgrms.)	(%)
1.	26. II. 1921	1.55	0.10	40	1918	4.79
2.	28. II. "	2.00	0.10	49	2390	4.88
3.	1. III. "	1.75	0.10	54	2276	4.22
4.	2. III. "	1.63	0.10	50	2248	4.50
5.	11. III. "	1.68	0.11	53	1998	3.76
6.	11. III. "	1.53	0.11	55	2620	4.76
7.	11. III. "	1.60	0.10	45	1592	3.54
8.	14. III. "	1.80	0.12	49	1256	2.56
9.	14. III. "	1.85	0.10	39	1329	3.41
10.	15. III. "	1.76	0.11	45	1552	3.44
Mean		1.72		48	1918	3.99

Of ten normal rabbits.

Body weight average 1.7 kilograms. (1.5-2.0 kilograms.)

Weight of liver " 48 grms. (39-55 grms.)

Glycogen in liver { " 1.92 grms. (1.26-2.62 grms.)
" 3.99 % (2.56-4.88 %)

Mr Fujii's estimation in January till April of this year (not yet published) as follows:

	19 normal rabbits	9 rabbits of one day's fasting	8 rabbits of two days' fasting
Glycogen in liver	1.953 grms. (0.551-	0.566 grm. (0.265-	0.325 grm. (0.131-
	3.465 grms.)	0.691 grm.)	0.681 grm.)
	3.49% (1.344-5.63%)	1.278% (0.602-1.608%)	0.863% (0.421-1.449%)

So, our estimation on normal rabbits agrees with each other very well. The estimation of Fujii on rabbits of one and two days' fasting can be utilized as the control of my cold-punctured rabbits, because the latters lived 10-42 hours after the puncture without taking any food.

Under 12 experiments, the cold-puncture hyperglycaemia was detected in 8 cases as follows:

EXPERIMENT III.

Date	Body weight (kg rms)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)	Urine					
						Quantity (c.c.)	Reaction	Specific gravity	Protein (%)	Sugar (%)	
18. I. 1921	1.75	Section of r. cervical sympathetic and r. auricular nerve.									
31. I. „	1.90	9.40 A.M.	0.12	38.9	11.0						
		10.20	Fettered.								
		10.22				25	alkaline	1014	—	0.012	
		10.23–10.32	Puuctured (10.30), bled a little, fell down to the left side after puncture.								
		10.34	Set free.								
		11.10	0.15	36.1	14.5						
		1.00 P.M.	0.13	33.8	17.0						
		3.00	0.14	32.7	18.0						
		5.00	0.13	32.5	19.0						
		11.00	0.11	32.4	14.5						
1. II. „	1.76	7.30 A.M.	0.12	29.0	8.0						
		10.30	0.14	28.0	14.5						
		1.30 P.M.	0.15	27.2	19.0						
		4.30	0.17	27.4	22.0						
		4.40	Killed with a blow.								
		Urine in the bladder.				30	acid	1032	0.1	0.132	

Liver { weight 45 grms.
glycogen content 647 mgrms.
1.439 %.

TABLE III.

The glycogen content in the liver of the normal rabbit whose body temperature descends under about thirty degrees.

No.	Date	Body weight (kgms.)	Initial		Hyperglycaemia due to puncture operation				Minimal blood sugar during experiment		Blood sugar at the body temperature under 30°C.				Hours after puncture		Duration of hyperglycaemia (hours)		Hours after puncture		Blood sugar when the rabbit was killed with a blow		Weight of liver (grms.)	Glycogen content of liver (mgms.)
			blood sugar (%)	body temperature (°C.)	Maximal blood sugar (%)	Body temp. at the max. p. s. (°C.)	Hours after puncture	max. p. s. (°C.)	Body temp. at the min. p. s. (°C.)	Hours after puncture	Body temp. at the min. p. s. (°C.)	Body temp. at the beginning of hyperglycaemia (°C.)	Maximal blood sugar (%)	Duration of hyperglycaemia (hours)	Hours after puncture	Body temp. (°C.)	when the rabbit was killed with a blow	Hours after puncture	Body temp. (°C.)	when the rabbit was killed with a blow	Hours after puncture	Body temp. (°C.)		
1	22. XII. 1920	1.95	0.12	38.9	0.14	35.2	1½	3½	30.1	0.12	6	28.8	0.22	9	18½	0.16	25.6	9	18½	0.16	25.6	50	832	1.62
2	21. I. 1921	1.57	0.10	39.8	0.15	37.7	2½	3½	34.9	0.10	7½	29.4	0.14	3	25½	0.11	25.6	3	25½	0.11	25.6	42	700	1.67
3	31. I. 1921	1.30	0.12	38.7	0.15	36.1	2½	3½	32.4	0.11	12½	28.0	0.17	6	30½	0.17	27.4	6	30½	0.17	27.4	45	647	1.44
4	1. II. 1921	1.50	0.11	39.1	0.17	36.0	1½	3½	35.2	0.10	7		0.12?		19	0.12	20.0		19	0.12	20.0	34	316	0.093
5	7. II. 1921	2.22	0.11	38.1	0.14	35.2	1½	3½	29.5	0.10	5½		0.11		11½	0.11	26.5		11½	0.11	26.5	72	2464	3.43
6	7. II. 1921	1.85	0.12	38.3	0.20	35.2	1½	3½	29.0	0.13	5		0.17		7	28.4	26.2		11½	0.17	26.2	59	982	1.62
7	10. II. 1921	2.23	0.12	38.4	0.23	36.2	1½	3½	30.1	0.15	4½		0.22	15	23	0.22	15.2		23	0.22	15.2	52	482.5	0.927
8	14. II. 1921	1.76	0.10	39.3	0.11	37.1	5½	3½	32.1	0.07	7½		0.17		32 < 40	28.6	26.8		42	0.17	22.0	51	70.8	0.139
9	23. II. 1921	1.67	0.12	38.6	0.19	33.0	3	3½	32.4	0.09	14		0.19	> 3	25	0.19	26.8		25	0.19	26.8	38	281.5	0.74
10	25. II. 1921	1.80	0.11	39.1	0.15	35.3	1	3½	29.1	0.12	10		0.19		11	28.0	28.0		11	0.19	28.0	41	562	1.372
11	28. II. 1921	1.71	0.10	37.9	0.20	34.6	1½	3½	29.7	0.14	3½		0.21	6	11½	0.17	27.8		11½	0.17	27.8	41	7.8	1.80
12	2. III. 1921	1.96	0.10	39.7	0.13	36.7	1½	3½	31.5	0.05	11½		0.08		13½	0.08	21.6		13½	0.08	21.6	53	74.5	0.141

The liver glycogen content of 7 rabbits (except No. 8, which will be explained hereafter) was on an average 0.644 gm. (0.2815-0.962 gm.), 1.36% (0.74-1.80%). So, it is about same with that of the rabbits of one day's fasting of Fujii.

EXPERIMENT VIII.

Date	Body weight (kgms)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)	Urine						
						Quantity (c.c.)	Reaction	Specific gravity	Protein (%)	Sugar (%)		
27. I. 1921	1.65	Section of r. cervical sympathetic and r. auricular nerve.										
14. II. "	1.76	3.30 P.M.	0.10	39.3	20.5							
		4.00	Fettered.									
		4.01-4.12	Punctured (4.10), bled a little, afterwards lay down to the right side.									
		4.13	Set free.									
		5.00	0.11	37.1	20.0							
		7.00	0.09	33.2	19.0							
		9.00	0.09	32.2	17.8							
		11.30	0.07	32.1	17.2							
		15. II. "	1.68	8.00 A.M.	0.10	29.6	10.0					
				10.30	0.11	28.8	14.6					
2.00 P.M.	0.11			28.6	17.7							
6.00	0.12			29.0	16.7							
9.00	0.08			29.7	16.2							
12.00	0.08			29.8	15.8							
16. II. "	1.55	8.30 A.M.	0.16	23.1	9.5							
		10.00	0.17	22.0	11.8							
		10.10	Killed with a blow.									
		Urine in the bladder.				35	acid	1026	0.15	0.096		
Liver		{ weight	51 grms.									
		{ glycogen content	{ 70.8 mgrms. 0.139 %.									

The liver glycogen content of rabbit No. 8 was small, in spite of the occurrence of hyperglycaemia. This rabbit showed an inclination to hypoglycaemia often in the course of the experiment and hyperglycaemia occurred first at a very low temperature. If the animal were killed somewhat earlier, it would have been one of the exceptions.

In the above table, there are four exceptional cases. In two cases of them, the liver glycogen content was remarkably small, while in the other two cases it was as much as in the cold-punctured ones with hyperglycaemia, or more. The notes of the former are as follows :

EXPERIMENT IV.

Date	Body weight (kgrms)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)	Urine				
						Quantity (c.c.)	Reaction	Specific gravity	Protein	Sugar (%)
18. I. 1921	1.45	Section of r. cervical sympathetic and r. auricular nerve.								
1. II. "	1.50	2.10 P.M.	0.11	39.1	20.0					
		2.45	Fettered.							
		2.47				30	neutral	1018	—	0.036
		2.50-2.58	Punctured (2.55), bled a little.							
		3.00	Set free.							
		4.10	0.17	36.0	22.0					
		6.01	0.15	35.6	20.0					
		8.10	0.10	35.4	18.5					
		10.10	0.10	35.2	18.0					
2. II. "	1.39	8.30 A.M.	0.12	21.3	13.0					
		10.00	0.12	20.0	16.0					
		10.10	Killed with a blow.							
		Urine in the bladder.				45	neutral	1012	±	4.432
Liver		{ weight	34 grms.							
		{ glycogen content	{ 31.6 mgrms. 0.093%.							

EXPERIMENT XII.

Date	Body weight (kgrms.)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)
28. II. 1921	1.80	Section of r. cervical sympathetic and r. auricular nerve.			
2. III. „	1.96	9.10 A.M.	0.10	39.7	12.0
		9.20	Fettered.		
		9.22-9.29	Punctured (9.28).		
		9.30	Set free.		
		10.00	0.13	36.7	14.0
		11.00	0.12	33.9	15.5
		1.00 P.M.	0.09	32.9	18.0
		3.00	0.08	32.8	19.0
		5.00	0.06	32.6	19.5
		7.00	0.06	32.5	17.0
		9.00	0.05	31.9	17.5
		12.00	0.06	30.9	16.5
3. III. „	1.73	9.00 A.M.	0.08	21.6	10.0
		9.20	Killed with a blow.		
Liver { weight		53 grms.			
{ glycogen content		{ 74.5 mgrms. 0.141%.			

Date	Body weight (kgrms)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)	Urine				
						Quantity (c.c.)	Reaction	Specific gravity	Protein (%)	Sugar (%)
17. I. 1921	1.63	Section of r. cervical sympathetic and r. auricular nerve.								
21. I. "	1.57	3.00 P.M.	0.10	39.8	23.4					
		3.10	Fettered.							
		3.12				5	acid		—	0.012
		3.15-3.22	Punctured (3.20), afterwards lay down to the left side.							
		3.23	Set free.							
		4.00	0.15	37.7	23.6					
		5.00	0.13	35.6	22.2					
		7.00	0.11	35.4	21.8					
		9.00	0.11	35.2	21.2					
		11.00	0.10	34.9	20.2					
22. I. "	1.45	8.30 A.M.	0.12	31.3	11.5					
		10.00	0.14	29.4	15.5					
		11.00	0.12	29.3	17.0					
		1.00 P.M.	0.12	28.1	18.5					
		3.00	0.11	26.7	20.0					
		4.30	0.11	25.6	21.2					
		4.40	Killed with a blow.							
		Urine in the bladder.				61	acid	1028	0.6	0.24
		Liver { weight 42 grms.								
		{ glycogen content 700 mgrms.								
		{ 1.67%.								

EXPERIMENT V.

Date	Body weight (kgms.)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)
21. I. 1921	2.40	Section of r. cervical sympathetic and r. auricular nerve.			
7. II. „	2.22	9.00 A.M.	0.11	38.1	9.5
		9.20	Fettered.		
		9.22-9.30	Punctured (9.28), bled a little, afterwards fell down to the left side.		
		9.32	Set free.		
		10.00	0.14	35.2	13.0
		11.00	0.12	33.0	15.0
		1.00 P.M.	0.10	30.7	17.5
		3.00	0.10	29.5	20.0
		5.00	0.11	29.2	19.0
		7.00	0.11	26.3	17.0
		9.00	0.11	26.5	16.5
		9.10	Killed with a blow.		
Liver		$\left\{ \begin{array}{l} \text{weight} \quad 72 \text{ grms.} \\ \text{glycogen content} \left\{ \begin{array}{l} 2464 \text{ mgrms.} \\ 3.425\% \end{array} \right. \end{array} \right.$			

The results of the experiments in this paragraph suggest that the negative result on the three rabbits in the former paragraph may be due to deficiency of the liver glycogen content, judging from the course of the blood sugar content. Under normal rabbits with the cold-puncture, the two cases Nos. 2 and 5 of tab. III must be looked upon as true exceptions. The negative result of the other cases is due to the insufficiency of the glycogen content of the liver.

Some of the negative cases in the experiments of Sakaguchi and co-workers,¹⁾ Sakaguchi²⁾ and Asakawa³⁾ seem to be clearly due to glycogen deficiency. But, some others of them should be looked upon as true exceptional ones as Nos. 2 and 5 of this paragraph.

M. Loewit⁴⁾ concluded, that the occurrence of the cold-glycosuria on frogs does not depend upon the liver glycogen content, because in negative cases the richness of the liver glycogen was proven and cold-glycosuria was produced on frogs with a nearly total removal of the liver.

So, while the "Fesselungs"⁵⁾ as well as ether⁶⁾ hyperglycaemia

1) K. Sakaguchi, I. Hayashi and S. Yezima, *Mitteil. med. Fakult. Univers. Tokyo*, **20** (1918), 78.

2) K. Sakaguchi, *Ibid.*, **20** (1918), 485.

3) O. Asakawa, *Ibid.*, **25** (1921), 539.

4) M. Loewit, *Arch. f. exp. Path. u. Pharm.*, **60** (1909), 29 and 424.

5) I. Fujii, *this Journal*, **2** (1921), 9.

6) I. Fujii, *Ibid.*, **2** (1921), 169.

occurred without fail on normal rabbits, the cold-puncture hyperglycaemia did fail to occur, though very seldom.

Diuretic hyperglycaemia occurs on normal rabbits usually, but not without exception. While the subcutaneous injection of magnesium sulphate caused also hyperglycaemia on normal cats in the majority of cases, ammonium chloride yielded a negative result not infrequently¹⁾. Not rarely diphtheria toxin and others fail to induce hyperglycaemia, except the experiments of S. Kuriyama²⁾ who failed, without exception, to find diphtheria toxin hyperglycaemia in rabbits. (See further L. Pollak³⁾) In such a case, the cause of the failure may be sought first in circumstances of resorption, etc.

The failure of the cold-puncture hyperglycaemia needs another explanation.

IV. THE MECHANISM OF THE "COLD-PUNCTURED" HYPERGLYCAEMIA AND GLYCOSURIA.

E. Pflüger suggested the essential cause of the cold-glycosuria which occurs by cooling of the body surface of animals to be a powerful reflex effect of the cooling of the skin upon the central nervous system.⁴⁾ The hyperglycaemia and glycosuria, which occur when rabbits body temperature falls by cooling, but is not less than about 30°C., undoubtedly can and must be explained by Pflüger's view, for hyperglycaemia and glycosuria can never be induced so long as the body temperature of the cold-punctured rabbits does not descend below about 30°C.

So, the experiments of H. Freund and F. Marchand⁵⁾ in which they produced hyperglycaemia in four rabbits out of seven (the rabbits were tied down) by cooling the abdominal wall with ether, whose body temperature descended two to five degrees and those of O. Asakawa⁶⁾, whose rabbits had the body temperature reduced to about 35° C. by a cold water bath, are good examples of Pflüger's explanation.

As a matter of cause, this cold-punctured hyperglycaemia-glycosuria can not be explained by the reflex effect of cooling.

An hypothesis was put forward by F. M. Allen⁷⁾, that the under-cooled blood itself acts as a direct stimulus to the nervous system, supported by the occurrence of glycosuria in frogs (Pflüger, Loewit) and its occurrence after removal of the liver (muscle diabetes) (Loe-

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- 1) Sachi. Morita (森田幸門), *Nippon-Naikwagakwai-Zasshi*, **8** (1920), 219. (Jap.)
  - 2) S. Kuriyama, *Journ. Biol. Chem.*, **34** (1918), 299.
  - 3) L. Pollak, *Arch. f. exp. Path. u. Pharm.*, **61** (1909), 376.
  - 4) E. Pflüger, *Arch. f. gesamt. Physiol.*, **118** (1907), 319 and *Das Glycogen u. seine Beziehung z. Zuckerkrankh.*, 2 Aufl. Bonn 1905, 528.
  - 5) H. Freund u. F. Marchand, *Arch. f. exp. Path. u. Pharm.*, **73** (1913), 276.
  - 6) O. Asakawa, *Mittell. med. Fakult. Univers. Tokyo*, **52** (1921), 550.
  - 7) F. M. Allen, *Studies concern. glycosuria and diabetes*, Boston 1913, 563.

wit). M. Loewit¹⁾ himself from his experiments agreed with the other view of E. Pflüger, that the cold-glycosuria is caused by the insufficient oxidation due to the lowering of the body temperature. R. Boehm and F. A. Hoffmann found first that in cats which were tied down on the holder, the body temperature lowered and finally when dead, the glycogen content of the liver and muscles showed a marked decrease.²⁾ And further, that when in cats the lower cervical cord was severed, the body temperature fell,—just as my cold-punctured rabbits,—but the glycogen content of the liver and muscles of the cats of this series of experiments, whose death was natural or was postponed a little by slightly warming them a while after the section, did not decrease compared with that of normal cats (not starved).³⁾ From these researches they draw the conclusion, that while on account of the intactness of the heat-regulating mechanism the body glycogen reserve was called upon to burn to prevent the body temperature fall in the first series of experiments, in the latter series, this mechanism lost its activity by the severing of the cervical cord and consequently the glycogen was not consumed abnormally.⁴⁾⁵⁾ Their opinion resembles that of Pflüger and Loewit. Boehm and Hoffmann discovered a large blood sugar content in the cats with the severed cervical cord after their death. They attributed it as a phenomenon in cadaver.

On the other hand, the cold-glycosuria might be attributed to the direct effect of cold on the liver itself.⁶⁾ This view is based upon the experiment of E. Masing⁷⁾, that the sugar content of a perfused solution from the extirpated rabbit liver increased when the solution was colder. But, the fact that whereas the diastase content of the liver of the rabbits, which were perfused with normal saline solution after haemorrhage, increased when the solution was colder, the rabbit liver *in vivo* behaved quite like others, lead the experimentalists to the idea that cold acts on the sugar center.⁸⁾

So, among several explanations concerning the cause of the cold-glycosuria, only Pflüger's view (reflex effect of cooling) is verified by a combination of the experiments of Freund und Marchand and Asakawa on one side and the discovery of the cold-puncture hypergly-

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1) M. Loewit, *Arch. f. exp. Path. u. Pharm.*, **60** (1909), 424.

2) R. Boehm and F. A. Hoffmann, *Arch. f. exp. Path. u. Pharm.*, **8** (1878), 375.

3) R. Boehm and F. A. Hoffmann, *Ibid.*, **8** (1878), 422.

4) R. Boehm and F. A. Hoffmann, *Ibid.*, **8** (1878), 430.

5) R. Boehm and F. A. Hoffmann, *Ibid.*, **8** (1878), 441.

6) H. Freund and F. Marchand, *Arch. f. exp. Path. u. Pharm.*, **73** (1913), 276.

7) E. Masing, *Arch. f. exp. Path. u. Pharm.*, **69** (1912), 431.

8) I. Bang, M. Ljundahl and V. Bohm, *Beitr. z. chem. Physiol. u. Path.*, **9** (1907), 428.

caemia on other side. But that this explanation cannot be adapted to the cold-puncture hyperglycaemia is self-evident.

1. The cold-puncture on the rabbit with bilateral splanchnectomy.

A rather similar experiment was already performed by R. Boehm and F. A. Hoffman.¹⁾ Cats were tied down on the holder and a section was made in the lower cervical cord. Then their body temperature lowered rapidly till 28.3–19.5°C. and they died finally. The liver glycogen content of these cats was as of normal (p. 422 of their essay). The blood sugar content was abnormally high. The average value of five cats was 0.46% (0.39–0.55%) (p. 430). The blood sugar content of their 23 normal cats was 0.15% (0.12–0.31) (p. 280) and that of three “Fesselungs”-diabetic cats 0.2%, 0.26% and 0.34% (p. 301). So, there was undoubted marked hyperglycaemia. They looked upon it as a cadaver phenomenon. The hyperglycaemia on the cats, in which the cervical cord was cut as usual, but the temperature fall was artificially prevented a little while, was not so marked (in four examples: 0.25% (0.21–0.30%).

The cold-puncture was carried out on the rabbits with bilateral splanchnectomy as follows :

TABLE
The blood sugar content resultant from the cold-

No.	Control experiment									
	Date	Body weight (kgms)	Initial blood sugar (%)	Maximal blood sugar after operation (%)	Hours after punct. to the max. blood sugar	Augmentation of blood sugar (%)	Date	Body weight (kgms)	Initial blood sugar (%)	body temperature (°C.)
1	30. XI. '19	1.77	0.12	0.12	$\frac{1}{3}$	—	1. XII. '19	1.78	0.12	39.5
2	30. XI. '19	1.85	0.11	0.12	$\frac{1}{3}$	0.01	1. XII. '19	1.98	0.12	39.6
3	10. I. '20	1.94	0.11	0.13	$\frac{1}{2}$	0.02	11. I. '20	1.97	0.11	38.9
4	20. I. '20	2.00	0.11	0.14	$\frac{1}{2}$	0.03	21. I. '20	1.97	0.12	38.6
5	23. I. '20	1.82	0.11	0.14	$\frac{1}{12}$	0.03	24. I. '20	1.86	0.10	38.6

1) R. Boehm and F. A. Hoffmann, Arch. f. exp. Path. u. Pharm., 8 (1878), 271 and 375.

The velocity and maximum of the body temperature fall was quite the same as in normal rabbits. The blood sugar content increased a very little owing to the operation itself (control and puncture), just as in the case of I. Fujii's "Fesselungs"-diabetes on bilateral splanchnectomized rabbits.¹⁾

And, when the body temperature dropped below 30°C. and further, there was no inclination to an increase of the blood sugar.

In comparison with its initial value, a minimum increase of the blood sugar below 30°C. of the body temperature was detected in three cases out of five. The excess was really 0.01-0.03%. It cannot be taken as an evidence of hyperglycaemia. Surely, such a small variation must be assumed as physiological. But if the variation of the blood sugar be clearly parallel to that of the body temperature, it should have some significance. Such a tendency could be traced only in one case (No. 5).

Albuminuria was detected, but not frequently. So, the cold-puncture hyperglycaemia and glycosuria is of central origin.

The so-called cold-glycosuria can be caused by two different mechanism: The one is a reflex of the cooling of the skin, as Pflüger pointed out. The other is the cold-puncture hyperglycaemia-glycosuria.

The cold-hyperglycaemia of Freund and Marchand and of Asakawa was induced solely by the first mechanism. If cooling depresses

IV.

puncture on the rabbit with bilateral splanchnectomy.

Principal experiment												
Maximal blood sugar after puncture (%)	Body temp. at the max. blood sugar (°C.)	Hours after punct to the max. blood sugar	Augmentation of blood sugar	Minimal blood sugar after puncture (%)	Body temp. at the minimal blood sugar (°C.)	Hours after punct to the min. blood sugar	Blood sugar at the body temperature under 30°C			Blood sugar before the rabbit is died (%)	Body temperature (°C.)	Hours after punct. until
							Maximal blood sugar (%)	Body temperature (°C.)	Hours after puncture			
0.13	37.9	$\frac{1}{4}$	0.01	0.10	26.8	24	0.10	29.0	31			
0.13	37.3	$\frac{1}{4}$	0.01	0.08	26.8	33 $\frac{1}{2}$	0.10	26.9	30			
0.13	37.7	$\frac{1}{8}$	0.02	0.11	32.2	4	0.12	24.9	10	13.7		21
0.15	37.7	$\frac{1}{4}$	0.03	0.11	31.3	4	0.14	27.4	10	17.4		22
0.13	36.0	$\frac{1}{2}$	0.02	0.09	29.0	10	0.13	29.8	4	14.3		27 $\frac{1}{2}$

1) I. Fujii, this Journal, 2 (1921), 56.

EXPERIMENT II.

Date	Body weight (kgms)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)	Urine				
						Quantity (c.c.)	Reaction	Specific gravity	Protein	Sugar (%)
22. XI. 1919	2.05	Splanchnectomy on both sides, and section of r. cervical sympathetic and r. auricular nerve.								
30. XI. "	1.85	12.10 P.M.	0.11	39.4	14.0					
		2.15	0.11	39.0	15.2					
		2.40	Fettered.							
		2.42				30	acid	1037	—	0.012
		2.44-2.50	Control operation.							
		2.51	Set free.							
		3.10	0.12	38.7	16.0					
		4.10	0.11	38.5	16.8					
		5.10	0.11	38.4	16.5					
		7.10	0.10	38.3	17.2					
		Appetite good.								
		Urine during the night.			50	alkaline	1038	—	0.012	
		2. XII. "	1.98	9.10 A.M.	0.12	39.6	13.2			
9.22	Fettered.									
9.24						20	alkaline	1041		0.024
9.25-9.30	Punctured (9.28), bled a little, afterwards lay down to the right side.									
9.31	Set free.									
10.00	0.13			37.3	15.2					
11.10	0.13			33.8	17.3					
11.50	Tonic convulsion, from this time turned to the left side.									
12.10 P.M.	0.13			32.2	18.1					
1.10	0.13			31.0	18.1					
2.10	0.13			30.5	18.1					
4.10	0.13			30.2	20.2					
4.25						60	neutral	1018	—	0.012
6.10	0.12	30.3	19.3							
8.10	0.10	30.3	18.8							
1. XII. "	1.81	8.40 A.M.	0.09	26.2	13.8					
		12.10 P.M.	0.10	24.4	17.5	15	alkaline		—	0.012
		3.40	0.10	26.9	20.8					
		7.10	0.08	26.8	19.6					
		Died in the night.								

the body temperature below about 30°C. as the experiments of Araki and others show, the other mechanism combines to it probably.

As already described in the previous chapter, the failure of several processes to cause hyperglycaemia-glycosuria may be due, sometimes, to

EXPERIMENT III.

Date	Body weight (kgrms)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)	Urine					
						Quantity (c.c.)	Reaction	Specific gravity	Protein	Sugar (%)	
14. XII. 1919	1.95	Splanchnectomy on both sides.									
8. I. 1920	1.95	Section of r. cervical sympathetic and r. auricular nerve.									
10. I. "	1.94	11.00 A.M.	0.11	38.2	16.5	20	acid	1022	—	0.012	
		12.00	0.12	38.6	18.8						
		12.10 P.M.	Fettered.								
		12.12									
		12.13—									
		12.18	Control operation.				30	acid	1017	—	0.012
		12.20	Set free.								
		12.30	0.13	38.7	18.8						
		1.30	0.12	39.1	19.3						
		2.30	0.12	39.2	20.4						
		3.30	0.12	39.6	20.7						
		4.25									
		5.30	0.12	39.3	20.2						
		7.30	0.11	39.3	18.3						
		Appetite good.				30	alkaline	1026	—	0.024	
11. I. "	1.96	8.30 A.M.	0.11	38.9	10.4						
		9.30	0.11	38.9	11.2						
		9.40	Fettered.								
		9.42									
		9.44—9.50	Punctured (9.46), afterwards crouched.								
		9.52	Set free.								
		10.00	0.13	37.7	11.8						
		11.00	0.12	34.4	12.3						
		12.00	0.11	32.2	13.2						
		1.00 P.M.	0.11	30.5	14.6						
2.00	0.11	29.8	14.8								
4.00	0.11	27.8	15.8								
6.00	0.12	26.3	16.5								
8.00	0.12	24.9	17.2								
10.00	0.11	23.8	16.8								
10.10				10	neutral						
12.00	0.11	23.4	16.8								
12. I. "	1.89	7.00 A.M.				10			0.3	0.228	
		7.10	Died.								

deficiency of the liver glycogen content. The rabbits with bilateral splanchnectomy were submitted to experiments for the first eight days after splanchnectomy i.e. after the nutrition was quite or nearly recovered. It may be not unreasonable, therefore, to assume their liver glycogen content as just normal.

But, on the one hand, I have not been able to find any report about the liver glycogen content of animal with bilateral splachnectomy and on the other hand strict control experiments to that of the previous chapter would seem to be necessary.

The liver glycogen content of the rabbit with bilateral splachnectomy was, therefore, estimated in two ways. In the first series, the rabbits whose splachnectomy was performed before eight days at least and whose nutrition was quite recovered, were killed suddenly with a blow on the neck. In the second series, the rabbits of the same conditions were cold-punctured and killed in the same way when their body temperature dropped below about 28°C.

TABLE V.

The liver glcogen content of the rabbit with bilateral splachnectomy.

No.	Date of splachnectomy	Body weight at the time	Date of experiment	Body weight on the day	Blood sugar	Weight of liver	Glycogen conten of liver	
		(kgrms.)		(kgrms.)	(%)	(grms.)	(mgrms)	(%)
1	18. II. '21	1.65	3. III. '21	1.65	0.09	40	562	1.41
2	18. II. '21	1.64	14. III. '21	1.40	0.11	42	874	2.08
3	9. III. '21	1.95	15. III. '21	1.88	0.10	57	2090	3.68
4	9. III. '21	1.78	16. III. '21	1.74	0.10	52	1589	3.06
5	6. III. '21	1.60	16. III. '21	1.68	0.11	46	884	1.92
	Mean	1.72		1.67		47.4	1200	2.43

Of five rabbits (the blood sugar content was normal):

Body weight   average 1.67 kilograms. (1.40-1.88 kilograms.)

Weight of liver   „   47.4 grms. (40-57 grms.)

Glycogen in liver { „   1.200 grms. (0.562-2.090 grms.)
                              „   2.43 % (1.41-3.68 %)

So, the glycogen content of these livers seems a little smaller compared with that of normal rabbits (see p. 423 of this essay.) But the minimum value of that of normal rabbits in Fujii's experiments and the small number of the experiments of this series would yet restrict us from drawing such a general conclusion.

EXPERIMENT I.

Date	Body weight (kg rms)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)	Urine						
						Quantity (c.c.)	Reaction	Specific gravity	Protein	Sugar (%)		
15. XII. 1920	2.00	Splanchnectomy on both sides.										
18. XII. "	1.70	Section of r. cervical sympathetic and r. auricular nerve.										
25. XII. "	1.83	4.10 P.M.	0.10	39.6	22.0							
		4.20	Fettered.									
		4.22				15	neutral		—	0.024		
		4.25-4.33	Punctured (4.31), afterwards fell down to the right side.									
		4.35	Set free.									
		6.40	0.10	35.8	19.5							
		8.40	0.10	31.8	18.0							
		11.10	0.10	29.5	18.0							
		26. XII. "	1.75	9.10 A.M.	0.10	20.7	10.0					
				11.10	0.10	19.0	11.5					
11.20						18?	acid		trace	0.024		
11.30	Killed with a blow.											
Liver		{ weight		49 grms.								
		{ glycogen content		{ 1059 mgrms. 2.28 %.								

EXPERIMENT V.

Date	Body weight (kgrsm.)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)
14. I. 1921	1.88	Splanchnectomy on both sides.			
21. I. „	1.90	Section of r. cervical sympathetic and r. auricular nerve.			
9. II. „	1.87	9.40 A.M.	0.12	38.8	13.5
		10.00	Fettered.		
		10.01-10.10	Punctured (10.09), afterwards fell down to the left side.		
		10.12	Set free.		
		10.40	0.12	36.2	15.5
		11.40	0.12	33.5	17.5
		1.10 P.M.	0.12	32.9	19.5
		3.10	0.12	33.2	20.5
		5.10	0.12	32.1	20.0
		7.10	0.11	30.1	18.5
		9.30	0.11	28.6	17.5
		9.40	Killed with a blow on the neck.		
Liver	{	weight	45 grms.		
		glycogen content	{ 422.5 mgrms. 0.946 %.		

EXPERIMENT VI.

Date	Body weight (kgrms.)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)
14. I. 1921	1.92	Splanchnectomy on both sides.			
20. I. „	1.92	Section of r. cervical sympathetic and r. auricular nerve.			
10. II. „	1.88	9.10 A.M.	0.11	38.7	12.5
		9.33	Fettered.		
		9.34-9.42	Punctured (9.41) afterwards fell down to the left side.		
		9.43	Set free.		
		10.10	0.13	35.7	15.5
		11.10	0.10	34.0	17.0
		1.10 P.M.	0.11	33.4	19.0
		3.10	0.09	32.7	19.5
		5.10	0.10	32.3	19.5
		7.10	0.11	32.2	18.0
		9.00	0.10	31.7	17.0
		11.00	0.09	31.8	17.0
		11.00	0.09	31.8	17.0
11. II. „	1.77	8.00 A.M.	0.10	—	8.0
		10.00	0.09	32.7	10.5
		12.00	0.10	31.2	12.0
		2.00 P.M.	0.10	30.0	13.2
		3.00	0.10	28.6	14.5
		3.10	Killed with a blow.		

Liver { weight 54 grms.
glycogen content { 1039 mgrms.
1.985 %.

TABLE VI.

The glycogen content in the liver of the rabbit with bilateral splanchnectomy whose body temperature descends under about thirty degrees.

No.	Date of splanchnectomy	Body weight on the day of experiment (kgms)	Date of experiment	Body weight on the day of experiment (kgms)	Initial blood sugar temp. (%)	Max. blood sugar due to operation (%)	Hours after punct. to the maximal blood sugar (°C.)	Min. blood sugar during the exper. (%)	Body temp. at the minimal blood sugar (°C.)	Hours after punct. to the minimal blood sugar (°C.)	Max. blood sugar at the body temp. under 30°C. (%)	Hours after sugar punct. until the rabbit was killed with a blow (°C.)	Blood sugar before the rabbit was killed with a blow (%)	Body temp. before the rabbit was killed with a blow (°C.)	Weight of liver (kgms)	Glycogen content of liver (mgms)	Glycogen content of liver (%)	Body weight on the day of slaughter (kgms)		
1	15. XII. 1920	2.00	25. XII. 1920	1.83	0.10	39.6	0.10	35.8	1	0.10	20.7	16½	0.10	18½	0.10	19.0	49	1059	2.28	1.75
2	16. XII. 1921	2.44	12. I. 1921	2.42	0.09	39.3	0.10	36.9	1	0.08	29.8	16½	0.08	16½	0.08	29.8	42	771	1.82	2.16
3	13. I. 1921	1.80	3. II. 1921	1.85	0.10	38.2	0.11	34.7	1	0.09	34.2	13	0.10	25	0.10	21.0	42	58.6	0.14	1.68
4	14. I. 1921	1.83	7. II. 1921	1.86	0.10	38.3	0.13	33.8	1½	0.09	24.7	9½	0.11	10½	0.11	24.0	54	879	1.63	
5	14. I. 1921	1.88	9. II. 1921	1.87	0.12	38.8	0.12	36.2	½	0.11	30.1	9	0.11	11½	0.11	28.6	45	425.5	0.95	
6	14. I. 1921	1.92	10. II. 1921	1.88	0.11	38.7	0.13	35.7	½	0.09	31.8	13½	0.10	29½	0.10	28.6	54	1039	1.98	1.77
7	13. I. 1921	1.80	12. II. 1921	1.75	0.11	38.6	0.12	36.4	½	0.08	27.7	22	0.08	24½	0.08	25.2	49	188	0.38	1.62
8	5. II. 1921	1.60	14. II. 1921	1.65	0.10	39.4	0.10	37.0	½	0.06	30.0	24	0.09	24	0.06	30.0	46	321	0.70	1.48
9	14. II. 1921	1.68	2. III. 1921	1.82	0.10	39.1	0.11	34.7	¾	0.10	24.6	6½	0.12	6½	0.10	24.6	48	773	1.61	
Mean	"		"	1.88													46.6	6127	1.27	

No hyperglycaemia was observed in them, though their body temperature dropped below 30° C. In three cases out of nine, the blood sugar content became subnormal. In No. 3, it became so temporarily. Also in four cases, the blood sugar content at the body temperature under 30°C. was higher, though very little.

The glycogen content of these livers was on an average 0.613 gm. (0.058–1.059 grms.), 1.27% (0.14–2.28%) and exceeded that of the normal cold-punctured rabbits on an average by 0.15 gm. If the cases in which the blood sugar content became subnormal in the course of the experiment be omitted, the difference is still greater [five cases: 0.835 gm. (0.426–1.059 grms), 1.69% (0.95–2.28%)].

This difference is sufficient to explain hyperglycaemia, though the small number of experiments prohibits the drawing of such a conclusion.

So, the failure of the cold-puncture hyperglycaemia-glycosuria on the rabbit with bilateral splanchnectomy is not due to the insufficiency of the liver glycogen content.

2. Is the activity of the nervous sugar mobilizing mechanism in the cold-punctured rabbit intact?

That the increase of the blood sugar content of the cold-punctured rabbit begins first when the body temperature lowers beneath about 30°C., while the cooling of rabbits induces hyperglycaemia when the temperature lowers only a little, verifies the hypothesis of Pflüger as already described in the previous chapter that a reflex effect of cooling of the skin causes the cold-glycosuria.

But, if taken very strictly, this conclusion can be admitted only under the condition that the activity of the nervous sugar mobilizing mechanism is normal in the cold-punctured rabbits.

The location of the central mechanism of the nervous sugar mobilization, the so-called "sugar center" is generally believed to be at a certain place in the medulla oblongata, according to the piqure of Bernard (E. Pflüger, O. Langendorff, J. J. R. Macleod, F. M. Allen, I. Bang, etc.).

On the contrary, some investigators would insist upon its localization in the upper part of the brain. M. Loeb¹⁾ from a clinical point of view guessed it to be near the pituitary body. By destroying a certain portion of the inter-brain of dogs, B. Aschner²⁾ produced a marked glycosuria and reduction of chromaffine substance in adrenal glands. These effects disappeared by the bilateral splanchnectomy.

1) M. Loeb, *Centralbl. f. inn. Med.*, 19. Jahrg. (1898), 893.

2) B. Aschner, *Wien. klin. Wochenschr.*, 1912, 1142.

The findings of H. Cushing and his co-workers^{1,2)} concerning the relation of the pituitary body to the carbohydrate metabolism have not been recognized as faultless, by later investigators. R. W. Keeton and F. C. Becht³⁾ concluded that hyperglycaemia induced by stimulation of the pituitary body depends upon the presence of splanchnic nerves.

K. Dresel and F. H. Lewy⁴⁾ would assume a higher center of the nervous sugar mobilization in the so-called nucleus periventricularis of the III. ventricle, which superintends that center in the medulla oblongata.

Thus, the hypothesis of localization of the sugar center anterior to the medulla oblongata is by no means predominant, but is not seldom renewed.

If now it is assumed provisionally that the so-called sugar center (of the first order—assumed) is located anterior to the medulla oblongata, it may be possible to suppose that the conduction from this center to the spinal cord was cut off by the cold-puncture and the cold-puncture hyperglycaemia was induced as the result of stimulation of the medullary or spinal sugar center (of the second order—assumed). Then, analogically to the various sensibilities of the respiratory centers of the two orders to the variation of hydrogen ion concentration in the blood, it may be supposed that the sugar center of the first order be more sensitive to a low temperature and consequently the cold-glycosuria of Asakawa and others may be due to the stimulation of the sugar center of the first order.

This supposition is undoubtedly carried too far. But, to justify the body temperature when the cold-puncture hyperglycaemia occurs, it is indispensable to know the integrity of the nervous mechanism of sugar mobilization.

For that purpose, ammonium chloride, which induces hyperglycaemia-glycosuria of the central mechanism, was used hypodermically. So, normal rabbits were cold-punctured and when the body temperature yet remained at 31–35°C., the 4 Mol solution of the salt was injected, at 0.5 gm. per kilogram body weight of animals.

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1) E. Goetsch, H. Cushing and C. Jacobsen, *Bull. Johns Hopkins Hosp.*, **22** (1911), 165.

2) L. H. Weed, H. Cushing and C. Jacobsen, *Ibid.*, **24** (1913), 40.

3) W. Keeton and F. C. Becht, *Amer. Journ. Physiol.*, **39** (1916), 109.

4) K. Dresel and F. H. Lewy, *Deutsch. med. Wochenschr.*, **47** (1921), 610.

EXPERIMENT IV.

Date	Body weight (kgms)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)	Urine				
						Quantity (c.c.)	Reaction	Specific gravity	Protein (%)	Sugar (%)
31 X. 1920	1.40	Section of r. cervical sympathetic and r. auricular nerve.								
4. XI. "	1.47	9.30 A.M.	0.11	38.0	11.0					
		10.00	Fettered.							
		10.03				5	acid		—	0.120
		10.04–10.13	Punctured (10.10), afterwards fell down to the right side.							
		10.15	Set free.							
		11.00	0.15	34.5	13.0					
		12.00	0.12	34.3	14.0					
		1.00 P.M.	0.11	34.2	15.0					
		1.15	Injection of 3.45 c.c. of salt solution.							
		2.00	0.18	33.3	15.0					
		3.00	0.20	33.4	15.5					
		5.00	0.09	33.6	16.8					
		7.20				7	acid		—	0.024
		8.00	0.12	34.0	16.8					
		11.00	0.13	34.3	16.2					
15. XI. "	1.37	8.30 A.M.	0.13	28.7	12.0					
		1.00 P.M.	0.15	28.0	12.0					
		8.00	0.12	30.0	20.0					
5. XI. "	1.34	9.00 A.M.	0.18	21.4	16.0					
		1.00 P.M.	0.22	21.2	18.8					
		8.30	0.18	22.0	20.0					
		11.00		20.8	19.5					
		Died in the night.								
		Urine in the bladder.				35	alkaline	1024	0.15	0.024

When the salt solution was injected, the blood sugar content was entirely or nearly recovered from the operation hyperglycaemia. Immediately after the injection, it increased remarkably and its maximum was reached in 2–3 hours, whereas there was no influence on the velocity of the temperature fall. (The body temperature of normal rabbits descends by the injection of this salt in some degrees; in extreme cases it was 30–26°C.¹⁾). The blood sugar then gradually decreased. In two cases in which the body temperature dropped below 30°C. on the day and night of the injection, the blood sugar content increased again at a

1) Ko. Naito, this Journal, 1 (1920), 131.

EXPERIMENT VI.

Date	Body weight (kgrms.)	Time	Blood sugar (%)	Body temperature (°C.)	Room temperature (°C.)
18. XI. 1920	1.94	Section of r. cervical sympathetic and r. auricular nerve.			
1. XII. „	2.02	9.00 A.M.	0.13	38.1	12.0
		9.20	Fettered.		
		9.22-9.32	Punctured (9.30), bled a large quantity, fell down to the right side after puncture.		
		9.35	Set free.		
		10.00	0.21	35.4	14.5
		10.30	Sit up straight, crouched.		
		11.00	0.15	35.2	15.5
		12.30	Fell down to the right side again.		
		1.00 P.M.	0.12	34.9	16.0
		3.00	0.13	34.3	19.0
		3.20	Injection of 4.7 c.c. of salt solution.		
		4.00	0.19	33.4	20.5
		5.00	0.27	32.3	20.5
		7.00	0.29	30.7	20.0
		9.00	0.24	30.8	19.0
		11.00	0.15	31.0	18.5
2. XII. „	1.76	8.30 A.M.	0.11	24.8	13.0
		12.00	0.09	23.3	18.5
		3.30 P.M.	0.10	23.7	22.0
		7.00	0.08	24.3	20.0
		11.00	0.08	24.5	20.5
		Died in the night.			

body temperature lower than 30°C. In the remaining cases the body temperature went below 30°C. in night and the blood sugar on the next day was subnormal, presumably owing to the diminution of the liver glycogen content by the salt injection.

By comparing these results with the ammonium chloride hyperglycaemia of Ko. Naito¹⁾ (His methods of drawing blood samples and of estimating blood sugar are differ from mine. The comparison is, therefore, approximate) and of me (not yet published) on normal rabbits, it can be safely concluded, that the hyperglycaemia by ammonium chloride on the cold-punctured rabbits is of quite the same course and degree as on normal rabbit.

So, the activity of the nervous sugar mobilizing mechanism in the cold-punctured rabbits is quite normal.

1) Ko. Naito, this Journal, I (1920), 131.

TABLE VII.

The blood sugar content of the cold-punctured rabbit when the ammonium chloride solution was injected hypodermically.

No.	Date	Body weight (kgms)	Initial		Max. blood sugar due to operation (%)	Body temp. at the maximal blood sugar (°C.)	Hours after punct. to	Blood sugar before the NH_4Cl -injection (%)	Body temp. before the NH_4Cl -injection (°C)	Hours after punct. till	Hyperglycaemia due to NH_4Cl -injection				Duration of hyperglycaemia (hours)	Minimal blood sugar after NH_4Cl -injection (%)	Lowest body temp. (°C.)
			Blood sugar (%)	body temp. (°C.)							Maximal blood sugar (%)	Body temp. at the max. b. s. (°C.)	Hours after injec. to the max. blood sugar	Hours after punct. to the max. blood sugar			
1.	3. XI. 1920	1.97	0.13	38.8	0.20	35.2	1	0.13	34.7	7½	0.21	33.9	1	8½	5	0.05	20.5
2.	4. XI. "	1.69	0.12	38.9	0.22	33.6	1	0.14	31.0	4	0.28	29.7	4	8	>25	0.21	20.8
3.	5. XI. "	1.80	0.11	39.4	0.19	37.5	½	0.13	34.7	3½	0.36	33.2	2½	6½	4	0.08	23.0
4.	14. XI. "	1.47	0.11	38.0	0.15	34.5	1	0.11	34.2	3	0.20	33.4	1½	5	2½	0.12	20.8
5.	16. XI. "	1.48	0.10	38.0	0.17	35.9	½	0.14	32.9	3½	0.24	32.2	2½	5½	5	0.05	23.7
6.	1. XII. "	2.02	0.13	38.1	0.21	35.4	½	0.13	34.3	5½	0.29	30.7	3½	9½	7	0.08	23.3
7.	1. XII. "	1.75	0.10	38.4	0.13	36.2	½	0.11	31.3	2	0.26	26.0	4½	7	>10	0.18	23.5

SUMMARY.

1. In the "Zwischenhirnstich" of E. Leschke, the incision between the inter- and midbrain was a little enlarged. The fall of the body temperature of the cold-punctured rabbit is so remarkably rapid and great that results of Leschke and others who adopted Leschke's original method have no comparison. This procedure may be named as the "cold-puncture." The reason is written in the text. The body temperature of my punctured rabbits falls to 33°C . in only about five hours; and finally it lowers to a little higher than the room temperature and varies with it. So, the result of the cold-puncture on the body temperature is quite the same as the extraction of the inter-brain of R. Isenschmid, while the process of cold-puncture is quite simple.

2. The blood sugar content of the cold-punctured rabbit, which recovers first to the normal value after the passing off of the operation hyperglycaemia, begins suddenly to increase as soon as the body temperature descends to about 30°C . and becomes greater, as the body temperature descends further. Glycosuria also occurs.

- (a) Failure of the cold puncture hyperglycaemia-glycosuria is due to the insufficiency of the liver glycogen content, except in a few cases.
- (b) The cold-puncture hyperglycaemia-glycosuria cannot be induced on the rabbit with bilateral splanchnectomy. So, it is one of central origin. The activity of the nervous mechanism of the sugar mobilization is intact on the cold-punctured rabbit.