Notes on Adrenaline Hyperglycaemia on the Rabbit. §

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In spite of the accumulation of information relating to adrenaline hyperglycaemia, even concerning conditions evoking hyperglycaemia by the drug there yet remain some thoroughly discordant testimonies which may puzzle the reader in forming a definite idea.

In an attempt to investigate the points at issue we have made a further study of this matter. Some results appear to be such interest as to justify a brief report. They consist chiefly of two parts, namely: (1) Comparison of the modes of application of the drug, that is of the subcutaneous administration and of the intravenous, upon the degree of hyperglycaemia and glycosuria, and (2) determination of the threshold of the total quantity of the drug in order to bring about hyperglycaemia.

Some data concerning the influence of aderenaline administration upon the epinephrine content of the suprarenal glands will be appended.

I. METHODS.

Male rabbits were exclusively employed. The feeding and treatments of the animals were carried out as usual in our Laboratory. Except for some rabbits, which were used in the infusion experiments of the pure saline solution, all the rabbits were first doubly splanchnectomized, and the cervical sympathetic nerve and the great ear nerve on one side were also divided. The actual experiment was carried out first two weeks after the division of the splanchnic nerves. Those animals, nutrition of which did not yet recovered, were not brought to the actual experiment.

§ The preliminary report of this paper appeared in Nippon Igakukwaishi, 1922, 6, 31.

As a rule, the blood samples were collected from the posterior branch of the vein of the denervated ear, and estimated for the sugar content by means of the micromethod of Bang (1920), and for the blood pigment by the method of Fleischl-Miescher. When a large volume of a diluted adrenaline solution was introduced intravenously, it was sometimes difficult to draw out the blood from the ear vein. In such cases, one carotid was laid bare and from its small branch the blood was taken. But, when no suitable small branch was found, the carotid itself was punctured by a fine syringe needle. In the mean time the cut end of the small artery branch or the needle hole on the carotid was clipped by means of a small artery forceps.

The adrenaline solution. The preparation of 1:1,000 solution of Sankyo & Co. was used as a full strength solution or diluted with 0.9% NaCl solution.

The full strength solution was administered either under the skin of the back or of the inner thigh, or in the great ear vein on the non-denervated side. The injection was made on the rabbit sitting loose in a large pan.

The adrenaline-saline solutions, varying in strength from 1:100,000 to 1:2,000,000, were introduced with a uniform velocity by means of a fine graduated bottle of Mariot with a diameter of 1.5 or 2.5 cms., in the outside branch of the external jugular vein of the fixed animal.

The urine was collected by catheterisation, and was determined for the sugar content by Bertrand.

Usually, the frequency of respiration of the animal was first noted, the blood samples were drawn, the urine was taken, and finally the anal temperature measured.

When the blood sugar experiment was finished, the animal was killed by a blow on the neck and the suprarenals were removed at once. The suprarenal gland on one side was used for the determination of epinephrine by means of Kodama's modification, and the opposite one was prepared for assay of the chromaffine substance by Negrin and Brücke.¹⁾

As the controls, the variation of the blood sugar content of the bilaterally splanchnectomized rabbit due to its being fixed for a long time was examined, and the results, given in the accompanying table, harmonize quite well with a previous report from our Laboratory.²⁾ By this manipulation, the blood sugar can increase, though very little, say, to 0.03%.

1) I. Fujii, Tohoku J. Exp. Med., 1920, 1, 44 f.

2) I. Fujii, Tohoku J. Exp. Med., 1921, 2, 56-63.

TABLE I.

Fixation experiments on the doubly splanchnectomized rabbits.

No.	Date	Body weight (kilos)	Blood sugar (%)		Urine sugar		Duration of fixation (hrs.)
			Before fixation	Maximum during fixation	Maximum during fixation (%)	mgram. per minute	
1	27. IV. '21.	1.93	0.10	0.14	0.05	0.02	5.5
2	28. IV. "	1.85	0.10	0.14	0.02	0.01	5.5
3	5. V. "	1.58	0.11	0.14	0.02	0.02	5.5
4	7. V. "	1.86	0.10	0.14	0.02	0.02	5.5
5	9. V. "	1.57	0.08	0.10	0.06	0.01	5.5
6	18. V. "	1.65	0.09	0.10	0.06	0.03	4.0

EXPERIMENTAL RESULTS.

In the first place the following problem frequently attacked by various writers will be dealt with :

Influence of the Modes of Application of Adrenaline upon the Degree and Duration of the Hyperglycaemia and Glycosuria.

In adhering to previous observers, the full strength solution of adrenaline hydrochlorid (1 : 1,000) was administered without dilution. The doses applied by them were commonly 0.1–0.3 c.c. per kilo of body weight; so in the present investigations 0.1 or 0.3 c.c. was dosaged. An intravenous application of a greater dose than 0.3 c.c. per kilo invariably proved fatal within a short time, while 0.3 mgrm. of the drug was tolerated by about half the number of rabbits experimented on.

Previously doubly splanchnectomized rabbits were exclusively applied. The general course of the experiments will be readily seen from the examples given in full. The quantity of sugar which appeared in the urine for unit time was calculated, since it is more important in judging the glycosuria or glycuressis than the concentration of sugar in the urine.³⁾ Roughly speaking, when the content of sugar in urine is found to be more than 0.1% or its total quantity per minute exceeds 0.1 mgrm., it can be considered as a sign of glycosuria.

The results are epitomized in Table II.

3) S. R. Benedict, E. Osterberg and I. Neuwirth, J. Biol. Chem., 1918, 34, 217.

EXAMPLE I.

24. III. '22. Room temp. 15-23°C. ♂ 1.72 kilos.

Adrenaline (1:1000): Subcutaneous injection.

(9. III. '22. Double splachnectomy.)

Time	Body temp. (°C)	Number of respiration	Urine				Blood sugar (%)
			Quantity (c.c.)	Reaction	Sugar		
					(%)	mgrm. per minute	
9:15	39.3	82		acid	0.031		0.10
	0.17 c.c. adrenaline (1 : 1000) hypodermically injected.						
:45	39.3	88	4.5	neutral	0.087	0.127	0.17
10:15	39.2	96	6.0	"	0.140	0.280	0.19
:45	39.1	96	5.0	"	0.832	1.387	0.21
11:15	38.8	98	5.0	"	1.409	2.415	0.28
:45	38.8	98	2.5	"	2.001	1.667	0.32
12:15	38.6	94	3.0	"	3.000	3.000	0.29
:45	38.8	94	2.0	"	3.721	2.481	0.27
13:15	38.9	88	2.0	"	4.167	2.722	0.25
14:15	38.9	80	2.0	"	1.967	0.657	0.22
15:15	38.9	72	1.5	"	1.082	0.269	0.19
16:15	38.8	72	1.5	"	0.428	0.107	0.17
17:15	38.8	74	1.0	"	0.091	0.015	0.15
18:15	38.8	72	1.0	"	0.041	0.007	0.12

Suprarenal glands: r. 0.212 grm. Boundary distinct. (Negrin)
 l. 0.231 grm. Epinephrine content { 0.213 mgrm. (Folin)
 { 0.092 %.

EXAMPLE II.

16. III. '22. Room temp. 18-22°C. ♂ 1.35 kilos.

Adrenaline (1:1000): Intravenous injection.

(26. I. '22. Double splachnectomy.)

Time	Body temp. (°C)	Number of respiration	Urine				Blood sugar (%)
			Quantity (c.c.)	Reaction	Sugar		
					(%)	mgrm. per minute	
10:50	38.9	180	6.0	alkaline	0.035		0.11
			0.14 c.c. adrenaline (1 : 1000) intravenously injected.				
11:20	38.9	220	2.0	alkaline	0.020	0.035	0.25
:50	"	280	2.0	"	0.061	0.041	0.26
12:20	"	126	2.0	"	0.275	0.183	0.30
:50	38.6	110	0.7	"	0.261	0.061	0.29
13:20	"	126	2.0	"	0.183	0.061	0.31
:50	38.5	128					0.26
14:20	"	126	1.5	"	0.122	0.031	0.26
:50	"	132					0.25
15:20	38.4	128	3.5	"	0.105	0.061	0.19
:50	38.5	120					0.18

Time.	Body temp. (°C)	Number of respiration	Urine				Blood sugar (%)			
			Quantity (c.c.)	Reaction	Sugar					
					(%)	mgram. per minute				
16:20	38.5	140	0	alkaline	0.008	0.040	0.12			
:50	"	124					0.12			
17:20	"	126					15.0	0.008	0.040	0.10
:50	"	118								0.09
18:20	"	106					0	0.09		
:50	"	118					0	0.09		
19:20	"	112	0	"	0.008	0.003	0.08			
:50	"	108	0				0.08			
20:15	"	108	2.0				0.09			
:50	"	106	0				0.09			

Suprarenal glands: r. 0.099 grm. Boundary distinct, (Negrin)
 1. 0.105 grm. Epinephrine content {0.192 mgrm. (Folin)
 {0.183 %.

TABLE

Infusion of adrenaline in full strength (1:1,000)

No.	Date	Body weight (kilos)	Quantity of adrenaline introduced (mgrm. per kilo)	Blood sugar			
				Before injection (%)	Maximum after injection (%)	Time to maximum (hrs.)	Time to recovering initial sugar content (hrs.)
1	21. I. '22.	1.60	Intravenously Hypodermically {	0.10	0.31	1½	5½
2	7. III. "	1.95		0.11	0.32	2	8
3	24. III. "	1.72		0.10	0.32	2½	9
4	21. I. "	1.70		0.11	0.31	1½	6½
5	9. III. "	1.63		0.11	0.35	1½	8
6	25. III. "	2.95		0.12	0.35	1½	8
7	12. III. "	1.97		0.11	0.33	3	9
8	16. III. "	1.35		0.11	0.31	2½	6½
9	17. III. "	2.13		0.13	0.28	½	9
10	16. III. "	1.57		0.10	0.42	3	10
11	20. III. "	2.60		0.12	0.27	1½	6
12	22. III. "	2.15		0.11	0.33	3	10

Whether the drug was administered either hypodermically or intravenously or whether further 0.1 c.c. for kilo or 0.3 c.c. was given occasioned no essential difference in the degree or in the duration of the hyperglycæmia; so generally speaking, the adrenaline administration was followed by an increase of the blood sugar content which reached the maximum,

about 0.3–0.4%, in half an hour to two hours and wore off in about seven to ten hours after the administration.

That the intravenous application of adrenaline acts far inferior in evoking the blood sugar increase as compared with the subcutaneous administration, has been insisted by several writers,⁴⁾ and really in the non-splanchnectomized animals. In the cases where small doses were used (0.1 mgrm. of adrenaline for one rabbit) no difference in the modes of application upon the glycaemic action was noted.⁵⁾ The report in which the doses were dissimilar according whether the drug was given directly into the blood or subcutaneously cannot be unconditionally utilised for the comparison of the efficacy of modes of application.⁶⁾

The data in the paper of Doi in relation to the dosage on the glycaemic effect of the intravenously injected adrenaline afford somewhat peculiar features; viz. the greater the dose of adrenaline, the smaller the effect.⁴⁾

II.

to the doubly splanchnectomized rabbits.

Urine						
Sugar					Quantity per minute	
(%)			mgrm. per minute			
Maximum after injection	Time to maximum (hrs.)	Time to recovering initial sugar content (hrs.)	Maximum after injection	Time to maximum (hrs.)	Maximum after injection (c.c.)	Time to maximum (hrs.)
4.54	4	7	8.33	3	0.77	$\frac{1}{2}$
4.32	4	—	6.32	3	1.00	$\frac{1}{2}$
4.17	4	9	3.00	3	0.20	1
4.00	3	7	23.81	2	1.00	2
4.52	3	$3\frac{1}{2}$	22.64	2	0.70	$1\frac{1}{2}$
4.77	$3\frac{1}{2}$	6	40.60	2	1.20	1
0.97	$1\frac{1}{2}$	7	0.22	1	0.16	3
0.27	$1\frac{1}{2}$	7	0.18	$1\frac{1}{2}$	0.50	7
2.46	$\frac{1}{2}$	7	0.29	$\frac{1}{2}$	0.13	1
0.12	$\frac{1}{2}$	—	—	—	—	—
0.41	5	7	0.10	5	0.05	5
0.55	5	7	0.09	5	0.05	$\frac{1}{2}$

4) L. Pollak, Schmiedeberg's Arch., 1909, 61, 158 ff.; I. Bang, Blutzucker, 1913 Wiesbaden, 89 ff.; Ri. Doi, Nisshin Igaku, 1919, 10, 93–95 & 128–134; A. L. Tatum, J. Pharm. Exp. Ther., 1921, 18, 125 ff.

5) I. Bang, (4), 93 f.

6) L. Pollak (4); P. Schenk and A. Heimann-Trosien, Ztschr. f. exp. Med., 1922, 29, 401.

The large quantity used by him was 0.6–0.8 mgrm. for rabbits weighing 1.6–1.83 kilos. It must be taken into consideration that circulatory disturbances follow an intravenous injection of a large dose of adrenaline, as Doi did.

In the present investigations only two kinds of dosage, viz. 0.1 mgrm. and 0.3 mgrm. respectively per kilo of body weight were employed. As no other small quantity was tried, the present data justify us in saying only that a dose of 0.1 mgrm. per kilo was sufficient to introduce the maximum increase of the blood sugar content.

In man 0.25 c.c. of 1:1,000 solution was given, from case to case, either subcutaneously or intravenously. The degree of hyperglycaemia was not materially influenced by the modes of application.⁷⁾

The occurrence of hypoglycaemia following the hyperglycaemic stage of adrenaline is not seldom remarked.⁸⁾ In the present twelve instances, however, it was never detected. In general, as several papers from our Laboratory indicate the doubly splanchnectomized rabbit inclines to betray the hypoglycaemic stage by the agents producing diabetes of the central mechanism. Our animals were previously bilaterally aplanchnectomized. Whether or not the interference of the splanchnic nerves is to be held responsible for the non-occurrence of the hypoglycaemic stage in our instances is open to question. It may possibly simply be due to the small number of animals used.

Contrary to the blood sugar, the output of the urine sugar was notably influenced by the modes of application of adrenaline, in complete accordance with former observations. The glycosuric action of adrenaline given subcutaneously was quite remarkable. Diuresis was also evident. The maximum of the glycosuria was 4% for both the doses, and 4–8 mgrms. per minute for the dose of 0.1 mgrm. per kilo and twenty to forty milligrams per minute for the dose of 0.3 mgrm. per kilo. The latter fact is accounted for in that the diuretic action was already wearing off or yet prevailing, when the sugar content of urine largely increased.

The intravenous injection of the drug, on the other hand, was associated with a very little glycosuria. The diuresis was quite small, especially in the large dose of adrenaline. The maximum sugar quantity excreted per minute or the maximum concentration of sugar in the cases of the intravenous injection was 1/10–1/40 respectively or 1/2–1/15 of the subcu-

7) H. L. Ulrich and H. Rypins, *J. Pharm. Exp. Ther.*, 1922, **19**, 215.

8) A. Erlandsen, *Biochem. Ztschr.*, 1910, **24**, 2 f.; I. Bang (4), 92; Brösamlen, *Mün. m. Woch.*, 1921, **68**, 965 (in man).

taneous for the dose of 0.1 mgrm. per kilo of body weight, and 1/200-1/400 respectively 1/8-1/40 for the dose of 0.3 mgrm.

Influence of Intravenous Injection of Diluted Adrenaline-Saline Solution upon the Blood Sugar Content.

To follow the natural output of epinephrine from the suprarenal glands the saline solution containing a minute quantity of adrenaline was intravenously introduced to the rabbit with a constant velocity, and an attempt was made to determine the total amount of the quantity as well as the velocity with which the solution is to be allowed to enter into the circulation, just necessary to cause the hyperglycaemia. As a matter of fact the continuous introduction of certain minute quantities of adrenaline for a unit of time is unquestionably proper and sufficient for the ultimate purpose, and not the dilution of adrenaline prior to the injection. But in most studies diluted solutions of adrenaline were employed.

A study of this kind was started from the Laboratory of Straub, henceforth several works done along these lines with improvements of experimental conditions were published one after another.

Any experimental condition, which can introduce an elevation of the blood sugar level by itself or in connection with other condition or conditions must by all means be avoided in this kind of experiments. Such are any kind of anaesthetic, fixation, etc., etc.

In the present experiments no narcotic was used; and for the sake of avoiding any possible influence of fixation of the animal the splanchnic nerves were previously dissected. Adrenaline solution of full strength (1 : 1,000) was diluted with 0.9% NaCl-solution, varying in strength from 1 : 2,000,000, 1 : 1,000,000, 1 : 500,000, 1 : 250,000 and 1 : 100,000.

It is generally believed that an injection of physiological saline solution into the circulation of rabbit does not result in hyperglycaemia. But, as the direct control of introduction of such a large quantity of the saline solution, some normal rabbits, to begin with, were fixed on the holder and exactly the same quantity of 0.9% NaCl-fluid as in the principal experiment, viz. 150-240 c.c. was injected into the external jugular vein, which was followed by hyperglycaemia and glycosuria of moderate strength as Table III shows. Therefore the normal rabbit cannot be utilized for this sort of experiment. On the bilaterally splanchnectomized rabbit the same kind of experiments yielded quite different results. The values did not substantially differ from those obtainable by the fixation alone, shown on Table I belonging to the preceding chapter.

TABLE

Infusion of 0.9% NaCl-solution to the normal

	No.	Date	Body weight (kilos)	0.9% NaCl-solution, introduced			Blood sug		
				Total volume (c.c.)	Volume per minute (c.c.)	Duration of infusion (hrs.)	Before infusion (%)	Maximum after infusion (%)	Sugar content reduced from Hb.-content (%)
Normal rabbits	1	5. X. '21.	2.42	210	2.0	1 $\frac{3}{4}$	0.12	0.22	0.29
	2	23. I. '22.	1.76	180	2.0	1 $\frac{1}{2}$	0.10	0.26	0.29
	3	23. I. "	1.50	180	1.5	2	0.11	0.21	0.25
Doubly splanchnecto- mized rabbits	4	20. VI. '21.	1.25	210	2.0	1 $\frac{3}{4}$	0.08	0.15	0.24
	5	22. VI. "	1.72	240	2.5	1 $\frac{1}{2}$	0.08	0.12	0.17
	6	27. VI. "	1.80	210	2.3	1 $\frac{1}{2}$	0.08	0.10	0.19
	7	30. VI. "	1.84	240	2.0	2	0.09	0.10	0.14
	8	12. VII. "	1.75	150	2.0	1 $\frac{1}{4}$	0.09	0.13	0.15
	9	22. VII. "	1.67	150	2.0	1 $\frac{1}{4}$	0.08	0.11	0.16

Intravenous injection of large volume of the saline solution causes an increase of the total quantity of the fluid in the circulatory system, so that the blood is diluted and the haemoglobin content decreases. In our cases the diminution of the latter to half of the initial was noted. If from the sugar value in the blood solution thus diluted that for the undiluted blood be calculated, the sugar content for the normal rabbits in the normal saline solution experiments would be 0.25–0.29% and for the bilaterally splanchnectomized ones 0.14–0.24%.

In the principal experiments the adrenaline saline solutions in strength of 1:2,000,000–1:100,000 were injected into the outside branch of the external jugular vein of the bilaterally splanchnectomized rabbits, with a velocity of 0.4–0.3 c.c. per minute, and actually so adjusted that the total quantity of the adrenaline hydrochlorid injected measured about 0.1–0.3 mgrm. per kilo of body weight. The dosage was, therefore, the same as in the experiments described in the former chapter, where the drug in full strength was given hypodermically or intravenously, and the total quantity of the fluid introduced seldom exceeded two hundred cubic centimeters.

For the injection of such a volume of the fluid about one and half hours were needed; after the injection the rabbit was freed.

As can readily be seen from an examination of Table IV, in almost all instances hyperglycaemia occurred. Only in those cases, in which the dose was not above 0.05 mgrm. per kilo, it was very doubtful. The excess

III.

and bilaterally splanchnectomized rabbits.

ar		Urine sugar					Haemoglobin content of blood		Body temperature	
Time to maximum (hrs.)	Time to recovering initial sugar content (hrs.)	Quantity per minute			Before infusion		Minimum after infusion		Before infusion	
		Maximum after infusion			Maximum after infusion		Maximum after infusion		Maximum after infusion	
		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(°C)	(°C)
3 1/2	—	2.02	1	1 3/4	2.02	1	17.0	10.4	38.9	35.7
1	—	1.09	2 1/2	—	3.63	2	18.1	17.0	37.9	35.9
3 1/2	—	0.69	4	—	0.18	4	19.5	15.5	38.2	35.5
1 3/4	—	0.05	1 3/4	—	0.47	2 1/2	15.2	7.7	38.7	33.7
1	—	0.11	3 3/4	—	0.10	2	17.2	11.6	38.8	36.7
1	—	0.05	3 3/4	—	0.06	2 1/2	17.9	9.6	38.6	33.6
1 1/4	—	0.05	3 3/4	—	0.09	2 1/2	16.1	11.7	39.1	37.5
3 3/4	—	0.02	3 3/4	—	0.03	2 1/2	15.6	8.8	38.2	37.2
3 3/4	—	0.18	3 3/4	—	0.12	2 1/2	16.2	11.1	38.3	35.0

of the blood sugar content due to adrenaline was 0.11–0.16%, mean 0.13% for the solution of 1 : 1,000,000; 0.03–0.15%, mean 0.1% for that of 500,000; 0.1–0.15%, mean 0.13% for that of 1 : 250,000, and 0.17–0.2%, mean 0.19% for that of 1 : 100,000. So, for the latter solution, i. e. the somewhat strong solution, the increase of the blood sugar content was clearly greater. But, in the cases of strong solutions of adrenaline the total volume of the fluid intravenously injected was far smaller than in the cases of the weaker ones; consequently the dilution of the blood must be smaller in the former cases, actually the haemoglobin content decreases but very little in the examples of the strong adrenaline solution (1 : 100,000). So, if these sugar values be recalculated for the non-diluted blood, they are 0.22% (0.19–0.24%) for the 1 : 1,000,000, 0.19% (0.14–0.34%) for the 1 : 500,000, 0.21% (0.16–27%) for the 1 : 250,000, and 0.23% (0.21–0.24%) for the 1 : 100,000. Practically no difference can be detected. And these values do not materially differ from those obtained by application of the full strength adrenaline solution (1 : 1,000), either subcutaneously or intravenously. Such a close agreement is remarkable, and probably cannot be regarded as merely accidental.

That a close relationship exists between the dilution of the blood i. e. the decrease of the haemoglobin content and the difference of the quantity

EXAMPLE III.

19. III. '22. Room temp. 10-22°C. ♂ 2.2 kilos.
1:100,000 adrenaline solution : Intravenous injection.

Time	Adrenaline saline solution		Body temp. (°C)	Number of respiration	Quantity (c.c.)	Urine		Blood		
	Total quantity (c.c.)	Adre- naline (mgrm.)				Reaction	Sugar		Haemo- globin content (%)	Sugar (%)
							(%)	mgrm. per minute		
10:20	Before operation		38.9	82		acid	0.037		16.5	0.09
	Operation									
10:35			38.3	88	5.0	”	0.037	0.122		0.10
:50	6.0	0.06	36.7	64	1.0	”	0.061	0.040		0.18
11:05	6.0	0.06	36.0	62	2.0	”	0.063	0.081		0.21
:20	6.0	0.06	35.2	62	4.0	”	0.375	1.000		0.25
:35	6.0	0.06	35.2	60	2.5	”	0.936	1.560		0.26
:50	6.0	0.06	35.0	62	3.5	”	1.110	2.590	15.2	0.29
12:20			34.6	60	6.0	”	1.924	3.881		0.28
:50			34.6	62	4.5	”	5.174	7.762		0.21
13:20			34.7	62	4.0	”	5.987	7.982		0.21
:50			34.7	60	2.5	”	3.986	2.661		0.20
14:20			34.6	64	2.5	”	0.400	0.167		0.18
:50			34.5	62		”			0.18	
15:20			34.5	60	3.5	”	0.052	0.062		0.16
:50			34.3	58		”			0.14	
16:20			34.3	59	2.0	”	0.061	0.020		0.14
:50			34.1	60		”			0.14	
17:20			34.0	58	4.0	”	0.046	0.031		0.13
:50			34.0	60		”			0.12	

r. 0.240 grm. Boundary distinct. (Negrin)

Suprarenal glands : 1. 0.289 grm. Epinephrine content { 0.241 mgrm. (Folin)
0.082 %.

of fluid introduced and the quantity of urine excreted, scarcely needs to be mentioned. The table shows it quite plainly. In general the quantity of urine, the concentration of sugar in the urine and the quantity of sugar excreted for an unit of time were quite variable in different cases; and no intimate relationship was to be found between them and the dose or the concentration of adrenaline introduced. Only when the adrenaline solution of 1:100,000 was administered the sugar content of the urine was definitely great. In these instances the total quantity of the injected fluid was much smaller and consequently the blood was diluted only a little.

There is no exact parallelism between the degree of the hyperglycaemia and that of the glycosuria. Only in the cases of 1:100,000 both the hyperglycaemia (in the diluted blood) and the glycosuria were remarkable, in comparison with the remaining instances. But, if the former be recalculated to the non-diluted blood, the degree of the blood sugar increase

does not differ from the rest.

The body temperature of the rabbits decreased during the experiment, but not very greatly. Such a decrease can be evoked even only by the fixation of the animal.

Further by these experiments we are justified in adhering to the view that the intravenous infusion of the dilute adrenaline solution can evoke the hyperglycaemia and glycosuria without collaboration of any narcotic, as urethane.⁹⁾ Contrary to some observers such as Gramenitzki, it was difficult to determine the threshold of the blood sugar content for the occurrence of glycosuria.

In the present experiments the adrenaline saline solution was introduced in $1\frac{1}{4}$ to 2 hours. Usually the highest point of the blood sugar content was already reached before the end of the infusion, and indeed in some instances soon after the beginning of the injection; but in about one third of the instances the blood sugar increased progressively even after discontinuation of the infusion, so that the highest level was reached in them in $1\frac{1}{4}$ – $1\frac{1}{2}$ hours or rarely $3\frac{1}{4}$ hour or more after cessation of infusion.

The postulation of the increase was not due to the hydraemia caused by the injection of a large volume of fluid, for it happened rather rarely with large volume of fluid, whereas the injection of a small quantity of the solution with a strength of 1:10,000 caused it frequently also. And further it is often noted that when the adrenaline solution of full strength is applied in a large dose the hyperglycaemia lasts a remarkably long time. The data in the preceding chapter also afford good examples. On the other hand it is a well substantiated fact that almost concurrently with the wearing off of the pressor action of an injected adrenaline it can no longer be detected in the general circulation even by a very sensitive biological method. Adrenaline very quickly loses its potency in the general circulation. Abundant confirmatory evidence has been yielded also in our Laboratory. In one example of Dr. Sugawara (personal communication) adrenaline hydrochlorid was intravenously injected into a cat, in a dose of about 0.09 mgrm. per kilo of body weight. Only up to about one hundred seconds adrenaline was discovered in the arterial blood by means of the rabbit intestine strip method. As a matter of fact, the larger the dose of adrenaline the longer it remains, without being destroyed, the length of time being far out of proportion to the dose.

While adrenaline as a vasoconstrictor agent, inhibiting agent for the

9) M. Gramenitzki, *Biochem. Ztschr.*, 1912, **46**, 200 ff.

TABLE

Infusion of dilute adrenaline saline solutions

No.	Date	Body weight (kilos)	Infused adrenaline solution							
			Dilution	Total quantity (c.c.)	Volume per minute (c.c.)	Total quantity of adrenaline (mgrm.)	Adrenaline per kilo (mgrm.)	Adrenaline per kilo per minute (mgrm.)	Duration of infusion (hrs.)	
1	6. XII. '21.	2.32	1:2,000,000	180	2.0	0.09	0.039	0.0004	1½	
2	13. XII. "	1.87		"	200	2.7	0.10	0.054	0.0007	1½
3	20. XII. "	1.59		"	200	2.7	0.10	0.062	0.0008	1½
4	27. I. '22.	1.73		"	200	2.7	0.10	0.058	0.0008	1½
5	8. XI. '21.	1.98	1:1,000,000	200	2.2	0.20	0.101	0.0011	1½	
6	29. XI. "	1.60		"	195	2.7	0.195	0.122	0.0016	1½
7	22. XII. "	2.13		"	180	2.0	0.18	0.084	0.0009	1½
8	25. I. '22.	2.02		"	150	2.0	0.15	0.070	0.0009	1½
9	13. VI. '21.	1.52	1:500,000	210	2.3	0.42	0.276	0.0029	1½	
10	14. VI. "	1.55		"	213	2.0	0.426	0.275	0.0026	1½
11	25. VII. "	1.56		"	180	2.0	0.36	0.231	0.0026	1½
12	27. VII. "	2.09		"	210	2.0	0.42	0.201	0.0019	1½
13	1. VIII. "	1.58	"	180	2.0	0.36	0.228	0.0025	1½	
14	3. VIII. "	1.62	"	180	2.0	0.36	0.222	0.0025	1½	
15	7. X. "	2.08	"	180	2.0	0.36	0.174	0.0019	1½	
16	3. XII. "	1.63	"	190	2.1	0.362	0.222	0.0026	1½	
17	15. XII. "	1.85	"	180	2.0	0.36	0.195	0.0022	1½	
18	15. VI. "	1.62	1:250,000	111	1.1	0.444	0.274	0.0026	1½	
19	23. VI. "	2.39		"	126	1.0	0.504	0.211	0.0017	2
20	28. VI. "	1.50		"	120	1.2	0.48	0.320	0.0035	1½
21	4. VII. "	1.45		"	90	1.0	0.36	0.248	0.0027	1½
22	6. VII. "	1.80	"	120	1.0	0.48	0.267	0.0022	2	
23	9. VII. "	1.77	"	120	1.3	0.48	0.270	0.0030	1½	
24	19. VII. "	1.50	"	110	1.2	0.44	0.290	0.0037	1½	
25	11. X. "	2.10	"	90	1.0	0.36	0.171	0.0019	1½	
26	14. III. '22.	1.75	1:100,000	20	0.27	0.20	0.114	0.0016	1½	
27	18. III. "	2.10		"	30	0.4	0.30	0.143	0.0019	1½
28	19. III. "	2.20		"	30	0.4	0.30	0.136	0.0018	1½
29	15. III. "	1.82		"	55	0.73	0.55	0.300	0.0040	1½
30	20. III. "	1.43	"	41	0.42	0.41	0.286	0.0038	1½	
31	24. III. "	1.58	"	45	0.6	0.45	0.285	0.0038	1½	

rabbit intestine movement, etc. has only a transitory existence in the general circulation, the hyperglycaemia due to adrenaline outlasts it a considerable length of time. As a matter of course, there is no ground for assuming that the excess of the blood sugar over its initial level must be so quickly annulled after cessation of the glycogenolytic cause. Injury to the heart or other tissues subsequent to a too rapid injection of adrenaline, by which Tatum¹⁰⁾ was inclined to explain a progressive increase of hyperglycaemia

10) A.L. Tatum. (4), 125 & 127.

IV.

to the bilaterally splanchnectomized rabbits.

Blood sugar					Urine				Haemoglobin content		Body temp.		Duration of experiment	
Before infusion	Maximum after infusion	Sugar content reduced from Hb-content	Time to maximum	Time to recovering initial sugar content	Sugar				Total volume	(%)		(C°)		
					(%)		mgm. per minute			Before infusion	Minimum after infusion	Before infusion		Minimum after infusion
					Maximum after infusion	Time to maximum	Maximum after infusion	Time to maximum						
(%)	(%)	(%)	(hrs.)	(hrs.)	Maximum after infusion	Time to maximum	Maximum after infusion	Time to maximum	(c.c.)	Before infusion	Minimum after infusion	Before infusion	Minimum after infusion	(hrs.)
0.09	0.13	0.16	$\frac{3}{4}$	1	0.10	$\frac{3}{4}$	0.39	$1\frac{1}{4}$	25.0	16.2	10.6	38.5	36.6	2 $\frac{1}{2}$
0.13	0.19	0.21	$\frac{1}{2}$	—	0.07	$\frac{3}{4}$	0.39	$\frac{3}{4}$	38.0	17.1	12.3	38.7	33.9	2 $\frac{1}{2}$
0.08	0.20	0.26	1	—	0.16	$1\frac{1}{4}$	1.93	$1\frac{1}{4}$	88.0	18.7	14.6	39.0	37.6	2 $\frac{1}{2}$
0.09	0.22	0.25	1	4 $\frac{1}{2}$	1.68	$1\frac{1}{4}$	16.20	$1\frac{1}{4}$	113.5	18.4	16.4	38.2	34.2	4 $\frac{1}{2}$
0.12	0.26	0.36	2 $\frac{1}{4}$	—	0.52	2	3.93	$1\frac{1}{4}$	102.5	19.4	13.6	38.3	37.4	2 $\frac{1}{2}$
0.10	0.21	0.31	2	—	0.42	$1\frac{1}{4}$	3.14	1	81.5	16.0	10.9	38.0	37.2	2 $\frac{1}{2}$
0.10	0.26	0.34	1	—	5.65	2 $\frac{1}{4}$	12.42	$1\frac{1}{4}$	101.0	17.6	13.8	38.5	35.5	2 $\frac{1}{2}$
0.12	0.24	0.31	$1\frac{1}{4}$	—	3.96	2 $\frac{1}{4}$	13.60	$1\frac{1}{4}$	92.0	18.7	14.1	38.3	34.2	4 $\frac{1}{2}$
0.10	0.16	0.38	1	—	0.15	$\frac{1}{2}$	0.16	$\frac{1}{4}$	9.8	17.5	5.4	38.3	33.2	1 $\frac{1}{2}$
0.09	0.12	0.25	$\frac{1}{4}$	—	—	—	—	—	—	15.5	6.8	38.0	31.8	2 $\frac{1}{2}$
0.07	0.22	0.41	$1\frac{1}{4}$	—	0.22	$1\frac{1}{4}$	2.38	1	59.0	17.5	9.4	38.6	34.0	1 $\frac{1}{2}$
0.11	0.24	0.24	2	—	0.37	2	16.80	$1\frac{1}{4}$	145.5	16.4	15.6	38.4	36.3	2 $\frac{1}{2}$
0.08	0.20	0.40	$1\frac{1}{2}$	—	0.15	2	2.02	$1\frac{1}{4}$	37.5	14.9	7.3	38.3	36.2	2 $\frac{1}{2}$
0.10	0.19	0.27	$1\frac{1}{4}$	—	0.17	1	2.05	$1\frac{1}{4}$	73.5	14.2	10.0	39.2	35.0	2 $\frac{1}{2}$
0.08	0.19	0.26	$1\frac{3}{4}$	—	0.52	$1\frac{3}{4}$	2.57	$1\frac{1}{4}$	38.0	16.1	11.2	39.0	34.6	2 $\frac{1}{2}$
0.14	0.25	0.26	$1\frac{1}{4}$	—	3.51	2	7.78	$1\frac{1}{4}$	155.5	15.7	15.2	38.8	37.0	2 $\frac{1}{2}$
0.09	0.19	0.30	$1\frac{1}{4}$	—	0.50	2	3.47	$1\frac{1}{4}$	70.0	13.2	8.0	38.9	37.0	2 $\frac{1}{2}$
0.11	0.25	0.29	2	—	0.78	2 $\frac{1}{4}$	2.76	2	26.0	16.2	13.4	38.7	34.0	2 $\frac{1}{2}$
0.08	0.24	0.35	$1\frac{1}{4}$	—	1.39	2 $\frac{3}{4}$	3.37	2	25.0	15.3	10.6	38.7	36.0	6 $\frac{1}{2}$
0.09	0.22	0.33	$\frac{1}{2}$	—	0.89	4 $\frac{1}{2}$	4.46	4 $\frac{1}{2}$	9.6	17.8	10.0	38.0	30.2	8 $\frac{1}{2}$
0.10	0.22	0.27	2	—	0.30	4 $\frac{1}{2}$	0.10	4 $\frac{1}{2}$	6.6	16.0	11.7	38.1	31.8	9 $\frac{1}{2}$
0.11	0.26	0.37	2	—	1.45	2 $\frac{1}{2}$	2.97	2 $\frac{1}{4}$	61.5	15.0	10.0	39.0	36.3	10
0.09	0.23	0.30	$\frac{3}{4}$	—	0.06	$\frac{1}{2}$	0.05	$\frac{1}{4}$	22.5	15.3	10.0	38.2	32.6	2 $\frac{1}{2}$
0.09	0.19	0.25	$1\frac{1}{4}$	—	2.29	$1\frac{1}{4}$	2.92	1	55.0	15.0	10.9	38.4	35.3	2 $\frac{1}{2}$
0.08	0.22	0.26	$1\frac{1}{4}$	—	0.15	1	0.37	1	15.0	14.3	10.7	38.8	35.2	2 $\frac{1}{2}$
0.10	0.30	0.34	$1\frac{1}{4}$	—	5.64	2 $\frac{3}{4}$	5.64	$1\frac{3}{4}$	31.0	17.4	16.2	38.6	35.4	6 $\frac{1}{4}$
0.12	0.31	0.35	2 $\frac{1}{4}$	—	4.77	$1\frac{3}{4}$	4.50	1	24.5	17.6	16.5	38.4	36.4	7 $\frac{1}{4}$
0.09	0.29	0.31	$1\frac{1}{4}$	—	5.99	2 $\frac{3}{4}$	7.98	2 $\frac{3}{4}$	47.5	16.5	15.9	38.9	34.0	7 $\frac{1}{4}$
0.11	0.29	0.33	$1\frac{1}{4}$	—	3.76	4 $\frac{1}{4}$	4.39	4 $\frac{1}{4}$	29.5	18.4	16.2	39.0	34.2	7 $\frac{1}{4}$
0.12	0.29	0.33	$1\frac{1}{4}$	—	4.41	4 $\frac{1}{4}$	1.13	4 $\frac{1}{4}$	8.0	18.0	16.0	39.3	33.2	7 $\frac{1}{4}$
0.10	0.29	0.32	$1\frac{1}{4}$	—	0.87	5 $\frac{1}{4}$	1.87	4 $\frac{1}{4}$	72.5	18.1	17.4	38.5	37.3	9

from shortly after the discontinuation of adrenaline infusion to an hour or more, in the experiments of Gramenitzki, may be only a subsidiary cause of this, or rather, to our minds, a matter of little importance.

From the data given in Table IV, it is not difficult to assume that a continuous infusion of adrenaline into the general circulation with a velocity of about 0.0008 mgrm. per kilo of body weight and per minute is capable of evoking hyperglycaemia.

The hyperglycaemia was invariably detected $1\frac{1}{4}$ hour after the be-

ginnig of injection, when the first blood sample during the infusion was, as a rule, drawn off.

According to Ritzmann,¹¹⁾ in order to induce glycosuria adrenline must be injected into the vein of the rabbit with the rate of more than 0.001 mgrm. per kilo and per minute. 0.002 mgrm. per kilo and per minute was the value given by Trendelenburg and Fleischhauer¹²⁾ for constant occurrence of hyperglycaemia. The former experimented on urethanized and bound rabbits, while ether was given only in the very beginning of the latter's experiments. The splanchnics were intact in the animals in both the reports. Recently Trendelenburg found another values, that is 0.00006–0.0001 mgrm. per kilo and per minute for constant occurrence of the hyperglycaemia alone, and 0.0001–0.0003 mgrm. for hyperglycaemia and nearly simultaneous appearance of glycosuria.¹³⁾

In a publication Trendelenburg assumed the rate of epinephrine output from the suprarenals of the rabbit at more than 0.00015–0.0002 mgrm. per kilo and per minute.¹⁴⁾ In the recent publication of the same writer epinephrine output of 0.0005 mgrm. or a little more in somewhat fifteen to twenty minutes after the sugar puncture was noted in two rabbits out of three.^{15*)}

The epinephrine output in the rabbits investigated in our Laboratory by means of the cava pocket method and the rabbit intestine strip method was 0.00004–0.00094 mgrm., mean 0.00035 mgrm. per kilo and per minute.¹⁵⁾ The animals were anaesthetized, nearly all of them by urethane. Such experimental conditions, as urethanizing, etherizing, fixing, laparotomy, etc., etc., cause unquestionably an increase of the blood sugar content. We term the epinephrine output in such conditions not simply as "spontaneous liberation," but always affix the term "so-called" before it.

Indeed, the problem as to what part is being played by the augmented epinephrine output, which usually or often occurs concurrently with the appearance of the hyperglycaemia of the central mechanism, for the increase of blood sugar has been frequently attacked, but still remains without being definitely answered.

11) W. Straub, M. m. W., 1909, 545; Ritzmann, Schmiedeberg's Arch., 1909, 61, 238.

12) P. Trendelenburg and K. Fleischhauer, Ztschr. f. ges. exp. Med., 1913, 1, 372 ff.

13) P. Trendelenburg, Pflüger's Arch., 1923, 201, 47–48 & *52.

14) P. Trendelenburg, Schmiedeberg's Arch., 1916, 79, 185.

15) Sakuji Kodama, Tohoku J. Exp. Med., 1923, 4, 232.

TABLE V.

Epinephrine content of suprarenal glands in the doubly splanchnectomized rabbits, in which adrenaline was administered.

No.	Date	Body weight (kilos)	Adrenaline solution		Weight of suprarenals		Epinephrine content		
			Dilution	Adrenaline per kilo (mgrm.)	r. (grm.)	l. (grm.)	Total quantity (mgrm.)	per kilo (mgrm.)	(%)
1	21. I. '22.	1.60	1:1,000 (hypodermically)	0.100	0.169	0.227	0.15	0.09	0.06
2	7. III. "	1.95	"	"	0.275	0.228	0.22	0.11	0.10
3	24. III. "	1.72	"	"	0.212	0.231	0.21	0.12	0.09
4	21. I. "	1.70	"	0.300	0.124	0.136	0.11	0.06	0.08
5	9. III. "	1.63	"	"	0.252	0.236	0.20	0.12	0.08
6	25. III. "	2.95	"	"	0.559	0.487	0.38	0.13	0.07
7	12. III. "	1.97	1:1,000 (intravenously)	0.100	0.196	0.213	0.21	0.11	0.11
8	16. III. "	1.35	"	"	0.099	0.105	0.19	0.14	0.18
9	17. III. "	2.13	"	"	0.243	0.300	0.29	0.14	0.12
10	16. III. "	1.57	"	0.300	0.085	0.104	0.13	0.08	0.13
11	20. III. "	2.60	"	"	0.304	0.386	0.21	0.08	0.07
12	22. III. "	2.15	"	"	0.312	0.295	0.22	0.10	0.07
13	14. III. "	1.75	1:100,000 (intravenously)	0.114	0.172	0.156	0.21	0.12	0.12
14	18. III. "	2.10	"	0.143	0.148	0.180	0.17	0.08	0.11
15	19. III. "	2.20	"	0.136	0.241	0.289	0.24	0.11	0.08
16	15. III. "	1.82	"	0.300	0.276	0.239	0.24	0.12	0.10
17	20. III. "	1.43	"	0.286	0.230	0.254	0.21	0.15	0.09
18	24. III. "	1.58	"	0.285	0.148	0.192	0.22	0.14	0.11
19	15. VI. '21.	1.62	1:250,000 (intravenously)	0.274	0.165	0.164	0.14	0.09	0.08
20	9. VII. "	1.77	"	0.270	0.171	0.200	0.17	0.10	0.09
21	19. VII. "	1.50	"	0.290	0.126	0.149	0.24	0.16	0.19
22	11. X. "	2.10	"	0.171	0.248	0.253	0.19	0.09	0.08
23	13. VI. "	1.52	1:500,000 (intravenously)	0.276	0.173	0.130	0.14	0.09	0.11
24	14. VI. "	1.55	"	0.275	0.229	0.193	0.18	0.12	0.08
25	25. VII. "	1.56	"	0.231	0.104	0.113	0.19	0.12	0.18
26	27. VII. "	2.09	"	0.201	0.174	0.180	0.17	0.08	0.10
27	1. VIII. "	1.58	"	0.228	0.173	0.190	0.16	0.10	0.09
28	3. VIII. "	1.62	"	0.222	0.130	0.137	0.23	0.14	0.18
29	7. X. "	2.08	"	0.174	0.253	0.263	0.15	0.07	0.06
30	3. XII. "	1.63	"	0.222	0.272	0.243	0.23	0.14	0.09
31	15. XII. "	1.85	"	0.195	0.193	0.187	0.19	0.10	0.10
32	8. XI. "	1.98	1:1,000,000 (intravenously)	0.101	0.305	0.348	0.24	0.12	0.07
33	29. XI. "	1.60	"	0.122	0.103	0.133	0.11	0.07	0.08
34	22. XII. "	2.13	"	0.084	0.452	0.417	0.30	0.14	0.07
35	25. I. '22.	2.02	"	0.070	0.290	0.306	0.21	0.10	0.07
36	6. XII. '21.	2.32	1:2,000,000 (intravenously)	0.039	0.267	0.242	0.20	0.09	0.08
37	13. XII. "	1.87	"	0.054	0.220	0.234	0.25	0.13	0.17
38	20. XII. "	1.59	"	0.062	0.199	0.174	0.26	0.16	0.13
39	27. I. '22.	1.73	"	0.058	0.181	0.250	0.25	0.14	0.10

The present results in combination with those on the so-called spontaneous liberation of epineprine may be taken as affording some suggestion to this question, though studies of this kind are yet too remote or too involved to give ultimate conclusions to the question.

Diuretic action of the diluted adrenaline saline solution occasioned the diuresis exceeding the urine volume in the rabbits into which was injected the large quantity of the pure saline fluid as the control. The blood sugar content prior to the appearance of glycosuria was 0.13–0.28%, mean 0.22%, in the cases of the non-occurrence of glycosuria, while it was 0.11–0.18%, mean 0.16%, when it was accompanied by diuresis. (If the values are reduced to the initial state of blood viz. to the non-diluted state, the numbers approach each other; that is 0.13–0.29%, mean 0.22%, and 0.17–0.21%, mean 0.19% respectively.)

Towards the end of the experiment the rabbit was killed by a blow on the neck and as soon as possible the suprarenal glands were removed, one of which was used for determination of epinephrine content by means of Kodama's modification and the other one for estimation of chromaffine substance by the procedure of Negrin and Brücke. The results on the epinephrine content are collected in Table V.

The data on the epinephrine content of the unilaterally splanchnectomized rabbits in a previous publication can be taken as the controls.¹⁶⁾

Roughly speaking, the results of the adrenaline experiments were not distinguishable in any way from the normal values on the denervated glands. Previous writers had detected no effect of adrenaline injection upon the epinephrine load of the suprarenals in normal animals.¹⁷⁾

The results of the chromaffine substance gave very similar evidence.

SUMMARY.

The present investigations were performed on bilaterally splanchnectomized rabbits.

1. Whether 0.1–0.3 mgrm. adrenaline in full strength (1:1,000) is administered to the rabbit hypodermically or intravenously, or whether 0.1 mgrm. or 0.3 mgrm. is dosaged for kilo of body weight has no bearing

16) So. Hirayama, *Tohoku J. Exp. Med.*, 1925, 5, 573.

17) T. R. Elliott, *J. Physiol.*, 1912, 44, 393 (cat); N. C. Borberg, *Skand. Arch. Physiol.*, 1913, 28, 139 (chrom. sub.) (rabbits; a little diminution in guinea-pigs.); Shige. Kuriyama, *J. Biol. Chem.*, 1918, 34, 305 (rabbits).

upon either the strength or the duration of hyperglycaemia.

As is well known, the hypodermical application of adrenaline brings about an intensive diuresis and glycosuria, while by the intravenous injection the urine secretion is greatly diminished and the sugar output in urine increases only a little.

2. An intravenous infusion of a large quantity of the dilute adrenaline saline solution, the total adrenaline quantity being dosaged as 0.1 mgrm. per kilo of body weight to 0.3 mgrm., without narcotic, also results in hyperglycaemia; if the blood sugar content in this series of experiments be calculated to the state of the initial haemoglobin content of blood, the dilution of the blood being taken into consideration, the maximum blood sugar content due to adrenaline does not materially differ from that yielded by the same dose of the drug in full strength (1 : 1,000).

3. The epinephrine load of the denervated suprarenal glands is not influenced by infusion of the dilute adrenaline saline solution.