DIASTASE CONCENTRATION IN THE BLOOD AND URINE OF NORMAL AND OF DIABETIC RATS

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In the course of a study devoted to the relation existing between pancreas and blood clotting[1], the diastase concentration in the blood and urine of normal rats and of rats in which diabetes had been provoked by means of alloxan, was investigated in some detail.

As the unexpectedly high concentration of the diastase in rat blood led to a modification of the technique used for its estimation, and also because in the literature widely diverging values are given for the proportion between the diastase concentration in the blood of diabetic and of normal individuals, it seemed worth while to publish our experience in full.

We used for our experiments rats that had received on two consecutive days an oral gift of 3 ml of a 20% dextrose solution followed after 45 minutes by a subcutaneous injection of 0.3 ml of a solution of alloxan in distilled water (75 mg alloxan per ml). The table gives the diastase concentration in the blood of 10 diabetic rats, measured by the amount of "dextrose" in mg obtained by the hydrolysis of glycogen, the amount of urine discharged in a period of one day by the animals 4–8, and of three days by the other ones, the absolute value of the sugar present in the urine and its concentration. The diastase concentration in the blood of 6 normal rats and in that of one animal (n. 11) in which the alloxan injection had failed to induce diabetes, serve for comparison. For the estimation of the diastase concentration dates were chosen that fell respectively 9, 16, 44 and 75 days after the alloxan injection.

The estimation of the *diastase concentration in the blood* was carried out according to the principle of BALTZER[2] and the directions given by DEKKER[3], but, because of the very high concentration of the diastase in rat blood, in a somewhat modified way.

Four wide test tubes received each 1 ml of a 0.9% NaCl solution, 1 ml distilled water, 0.1 ml of a saturated NaF solution, and finally 0.1 ml blood. The latter was taken from the tail of a rat by means of a pipette. Two of the tubes served as controls, but to the two other ones 9 ml of a freshly prepared 0.3% glycogen sol were added. All four were immediately afterwards placed in a waterbath at 38° C, in which they remained for two hours. After that the tubes were cooled in ice water. To the two control tubes now also 9 ml glycogen sol were added, and then they received all four 1 ml 0.1 n NaOH and 5 ml of a 0.45% solution of $ZnSO_4$, and were heated for a few minutes in boiling water. In this way the proteins were precipitated, and the enzyme activity was brought to an end. In the two hours during which the tubes remained in the water bath the blood diastase had hydrolysed part of the glycogen; the reducing substances that were formed in this way, were estimated at the conclusion of the experiment according to the method of HAGEDORN and JENSEN[4]. To this end we did not use the total amount of the filtrate obtained from the 17.2 ml fluid in each tube, but only 1 ml, for that appeared to be sufficient.

For the dextrose estimation according to HAGEDORN and JENSEN instead of potassium ferricyanide made alkaline by the aid of carbonate, the ferricyanide reagent recommended by FUJITA

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and OKAMOTO[5] was used. The latter consists of 1.65 g potassium ferricyanide, 174 g sec. potassium phosphate and 11.2 g KOH dissolved in distilled water, and brought to a volume of 1 liter; it is to be kept in a dark bottle. On this reagent 5 ml is added to 1 ml of the filtrate mentioned above. The tubes remain for 15 minutes in boiling water, and are then for as many minutes cooled in cold water. (If the mixture loses its yellow colour when it is boiled, this means that not enough oxydiser is present, and then more potassium ferricyanide has to be added. This is done in gifts of 2 ml, after which the mixture is once more to be boiled for 15 minutes. The treatment is to be repeated untill the yellow colour persists. In our estimations. however, in which but 1 ml filtrate was used, the original amount of oxydiser was always sufficient).

After the mixture has been cooled, the remaining oxydiser is estimated. To this end use is made of a mixture of KJ, $ZnSO_4$ and NaCl. This is obtained by mixing equal amounts of the following two solutions; solution I consists of 5 g KJ and 25 g NaCl dissolved in distilled water and brought to a volume of 1 liter; the precipitation of iodine is prevented by the addition of a drop of mercury and by keeping the solution in a dark bottle; solution II consists of 10 g $ZnSO_4 \cdot 7H_2O$ and 25 g NaCl dissolved in distilled water and brought to a volume of 100 ml. An amount of 7.5 ml of the mixture and 5 ml diluted HCl (1 vol. concentrated HCl diluted with 5 vol. distilled water) are added to the contents of each tube, and after the fluids have thoroughly been mixed, the mixture is titrated with 0.005 n thiosulphate. Amylum dissolved to an amount of 1 % in a concentrated NaCl solution serves as indicator. (If ferricyanide solution has been added to restore the surplus of oxydiser, for every 2 ml that have been used 3 ml of the mixture of KJ, $ZnSO_4$ and NaCl and 2 ml diluted HCl should be added).

As 1 ml 0.005 n ferricyanide solution and the equivalent amount of thiosulphate solution (i.e. 1 ml of the 0.005 n solution) correspond with 0.174 mg dextrose, and as of the original 17.2 ml fluid in the tubes always but 1 ml was used for the estimation, the amount of "dextrose" in mg that under the conditions of the experiment would be formed from the glycogen by the amount of diastase present in 100 ml blood, must be (B — A) 17.2·1000·0.174, B and A being the amounts of the 0.005 n thiosulphate solution in ml required for the titration of the mixtures without and with glycogen hydrolysis (cf. DEKKER[3]), pp. 267 and 164). The values for B and A themselves are the averages of estimations made in duplo.

The estimation of the *diastase concentration in the urine* was carried out either with the ordinary method of WOHLGEMUTH[6], in which use is made of the diastase index $d_{15}^{45°}$ or else with the more accurate technique described by HALLMANN[7].

The average amounts of "dextrose" found by substituting the values obtained for B and A in the formula (B-A) 17.2·1000·0.174, which, as indicated above, serve as a measure for the diastase concentration in the blood, were for 6 normal rats 6783 mg, for 10 rats in which by means of alloxan diabetes had been induced 6509 mg, and for 1 rat in which the alloxan injections had failed to provoke diabetes 6763 mg. The amount of "dextrose" produced by the same volume of normal human blood, varies between 190 mg and 310 mg (see Table I).

The differences between the amounts of diastase found in the various groups of rats remain within the range of the probable error, and there is therefore, no matter how strongly the intensity of the sugar excretion varies, and no matter how short or how long the time that elapsed between the induction of the diabetic condition and the determination of the diastase concentration, no difference between the values found for the latter in the blood of normal and of diabetic rats.

With regard to the diastase concentration in the urine of diabetic, alloxan-resistant and normal rats there is no difference either, provided that the dilution of the urine in the diabetic rats — the latter are all suffering from polyuria — is taken into account. To this end the amount of "dextrose" that serves as a measure for the diastase concentration is multiplied by the amount of urine that is discharged on the day of the estimation the products prove to be practically identical: the average values appeared to be fo normal rats (4 estimations) 386, for rats in which alloxan had failed to induce diabetes (10 estimations) 402, and for diabetic rats (27 estimations) 362.

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TABLE I

Number of test animals	Urine				Number of days between alloxan injection and diastase estimation	Mg "dextrose" obtained from glycogen by diastase present in 100 ml blood
	FEHLING'S solution	Amount of urine in ml in 1 or 3 days	Percentage of sugar	Amount of sugar secreted in 1 or 3 days		
1	+	3 days: 149	3.6	3 days: 5.3 g	44	6201
2	+	,, 185	4.0	,, 7.4 g	44	5662
3	+	,, 240	4.7	,, 11.3 g	44	5782
4	trace	1 day: 11	—	—	75	6794
5	+	,, 54	7.9	1 day: 4.3 g	75	6463
6	+	,, 41	9.1	,, 3.7 g	75	6405
7	— (control)	,, 6	—	—	—	5896
8	— (control)	,, 5	—	—	—	6285
9	+	3 days: 105	8.6	3 days: 9.0 g	9	5777
10	+	,, 82	8.1	,, 6.6 g	9	7840
11	neg.	,, 29	0	,, 0 g	9	6763
12	— (control)	not determ.	—	—	—	6974
13	— (control)	,, ,,	—	—	—	6974
14	+	3 days: 73	9.3	3 days: 6.8 g	16	7970
15	+	,, 244	10.5	,, 25.6 g	16	6198
16	— (control)	not determ.	—	—	—	7329
17	— (control)	,, ,,	—	—	—	7237

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SUMMARY

1. The estimation of the diastase concentration in the blood of normal rats, rats in which diabetes had been induced by means of alloxan, and in a rat that proved to be resistant against alloxan, gave practically identical values.

2. Similar estimations of the diastase concentration in the urine also failed to reveal differences, at least when the dilution of the urine owing to the polyuria of the diabetic animals, is taken into account.

3. The high concentration of the diastase in the rat blood required some modification in the technique of the estimation, of which details are given.

RÉSUMÉ

1. Les teneurs en diastase du sang de rats normaux, de rats rendus diabétiques par l'alloxane, et d'un rat résistant à l'action de l'alloxane, sont pratiquement les mêmes.

2. La même observation s'applique à l'urine, à la condition de tenir compte de la dilution du liquide due à la polyurie des animaux diabétiques.

3. La teneur élevée en diastase du sang du rat, nécessite une modification, décrite ici, de la technique habituelle du dosage de cet enzyme.

ZUSAMMENFASSUNG

1. Die Bestimmung des Diastasegehaltes im Blut normaler, nach Applikation von Alloxan diabetischer Ratten und einer Ratte, die gegen Behandlung mit Alloxan resistent war ergab praktisch identische Werte.

2. Analoge Diastasebestimmungen im Urin zeigten ebenfalls keine Abweichungen, falls dabei die Verdünnung des Urins durch die den diabetischen Ratten eigene Polyurie mitberücksichtigt wurde.

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3. Der hohe Diastasegehalt des Rattenblutes erforderte eine Modifikation der Bestimmungsmethodik, worüber Besonderheiten mitgeteilt werden.

REFERENCES

[1] E. HECHT, *Acta med. Scand.*, in press.
[2] F. BALTZER, II. *Mitt. Klin. Wschr.* 14 (1935) 1395.
[3] W. A. L. DEKKER, Leiden 1940.
[4] H. C. HAGEDORN AND B. N. JENSEN, *Bioch. Zschr.*, 135 (1923) 46.
[5] A. FUJITA AND K. OKAMOTO, *Bioch. Zschr.*, 225 (1930) 368.
[6] WOHLGEMUTH, see 3, p. 80.
[7] L. HALLMANN, Leipzig 1941.

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