Acta Medica Okayama

Volume 17, *Issue* 4 1963 *Article* 1

AUGUST 1963

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Abstract

Using the method given by GOLDSTEIN (1961)9 for the determination of serum beta-glucuronidase activity, this value was determined in both normal and patients with epilepsy, neuroses, psychoses and multiple sclerosis. Of the patient groups examined, the group of those suffering from epilepsy is the only one showing any difference of statistical significance for all four methods of determination. The group of patients suffering from neuroses differs significantly from the normal group as regards the results got by the method of heat coagulation for removal of the proteins. The material is however too small to provide any explanation of the results, but it appears to show that a determination of serum glucuronidase activity may be of interest in groups of diseases other than malignant tumors.

*PMID: 14123338 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 17, 175—182 (1963)

SERUM BETA-GLUCURONIDASE DETERMINATION IN NORMAL SUBJECTS AND IN NEUROLOGICAL AND MENTAL PATIENTS, A PROVISIONAL REPORT

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Received for publication, August 12, 1963

In recent years, serum beta-glucuronidase determinations have been employed by various workers in the diagnosis of cancer of the breast and of the female genitals (COHEN *et al.*, 1951[1,2], ODELL *et al.*, 1949[3], FISHMANN, 1947[4], FISHMANN *et al.*, 1951)[5,6]. In particular, a study has been made of the changes which occur in the enzyme activity in oestrogen therapy. As the determination does not appear to have been employed in the investigation of other disease groups, it was decided to include the procedure as a step in a series of studies on multiple sclerosis.

TALALAY (1946)[7] introduced phenolphthalein-mono-beta-glucuronide as a substrate in the determination of serum beta-glucuronidase. The enzymatic splitting of this substance results in a pink colour which can be measured by spectrophotometry in alkaline solution. There is no actual deproteinisation in this method, and the protein precipitated during the analysis is removed by centrifugation before making the actual reading. This method has been discussed by various workers, with particular reference to whether a deproteinisation is involved or not.

FISHMANN *et al.* (1948)[8] used trichloracetic acid as a deproteinising agent. In a study of 16 normal subjects, these workers found an enzyme activity varying from 0~230 units/100 ml serum, with a mean value of 101 units. This value is lower than the values found by GOLDSTEIN (1961)[9] and also lower than in the normal material presented here. Further, these workers showed that there are not very great amounts of beta-glucuronidase in erythrocytes, but a high concentration in both granulocytes and lymphocytes. The presence of the enzyme is also demonstrated in saliva, gastric juice, tears, urine and the cerebrospinal fluid.

FISHMANN *et al.*, 1950 (s) modification found extensive application, but a few workers (Moore *et al.*,[11] 1950; Mills *et al.*,[10] 1953) demonstrated that the precipitated protein binds some of the coloured substance, so that lower values are obtained.

GOLDSTEIN (1961)[9] discusses a number of studies of these observations, and

finds that while high values are obtained provided the proteins are not precipitated (I) or are precipitated by acetone (IV), considerably lower values are got if the proteins are precipitated by trichloracetic acid (II) or by boiling (heat coagulation) (III).

The methods given by GOLDSTEIN (I-IV) have been followed in the present study, and beta-glucuronidase determined in the four experiments set up in parallel.

The material includes a normal material of 8 men and 18 women, 12 patients suffering from multiple sclerosis, 8 patients with epilepsy, 7 with mental diseases and 14 with neuroses.

The patient material is not selected, but taken as representing an average of these groups, with the exception of the 12 patients with multiple selerosis, who all come from the Sanatorium for the Treatment of Multiple Sclerosis, Haslev.

METHODS

This is practically identical with that given by GOLDSTEIN[9].

Acetate buffer solution, 0.1 *M*. To make pH 4.0 solution, sodium acetate ($CH_3COONa \cdot 3H_2O$) 2.028 g and glacial acetic acid 4.87 ml, and for pH 4.5 solution 5.785 g and 3.25 ml respectively, are diluted to 1,000 ml and adjusted on pH meter.

Substrate : phenolphthalein mono-beta-glucuronic acid, 0.01 *M*. "Sigma" reagents are used in the preparation following the instructions supplied (Sigma Bulletin, 105).

Glycol buffer, 0.4 *M*, *pH* 10.55. 16.30 g glycol and 12.65 g sodium chloride are dissolved in water, 10.9 concentrated NaOH is added and the volume diluted to 1,000 ml. Adjust on pH meter.

Glycol buffer-alkali mixture. 200 ml glycol buffer is mixed with 100 ml 0.25 *N* NaOH.

Trichloracetic acid, 5 *per cent*. 1 ml phenolphthalein reagent, 250 μg in 80 per cent alcohol.

Procedure :

In two test-tubes, place 0.6 ml haemolysis-free serum plus 4.8 ml of 0.1 *M* acetate buffer, pH 4.5. Add 0.6 ml of substrate to the one tube, stopper the tubes and place them in a water-bath at 37°C for 24 hours, remove them from the water-bath and then add 0.6 ml substrate to the other tube (control).

These samples are now used *immediately* for the following four analyses :

I. Proteins not removed : Pipette 1 ml sample and 1 ml control into two centrifuge tubes. Prepare a standard consisting of 0.1 ml phenolphthalin standard, 0.8 ml 0.1 *M* acetate buffer pH 4.5 and 0.1 ml distilled water, together with a

blind sample, analogous with the standard, except that the phenolphthalein solution is replaced by 0.1 ml distilled water. Readings are made, including the blind sample, in a Beckmann DU spectrophotometer at 540 mμ.

II. Proteins removed by trichloracetic acid : One sample and 1 ml control are pipetted into two centrifuge tubes containing 1 ml of 5 per cent trichloracetic acid, the contents then mixed and centrifuged. The supernatant is decanted into a fresh centrifuge tube. The sediment is washed with 0.5 ml distilled water, centrifuged, and the supernatant decanted to the remainder of the sample. Washing is repeated. Standard and blind are prepared as described under I, and supplemented further with 1 ml of 5 per cent trichloracetic acid. Now add 4 ml glycol-alkali mixture to all tubes, and as described in I.

III. Proteins removed by heat coagulation : Two ml of sample and control in centrifuge tubes are immersed in boiling water for 1 minute. They are then centrifuged and 1 ml of the supernatant treated as described in I.

IV. Proteins removed by acetone : To 2 ml of sample and control (i. a. the remainder in the tubes) add 2 ml acetone, mix, centrifuge, transfer 2 ml of the supernatant to clean tubes. Standard and blind sample are prepared as described in I, and 1 ml acetone added. Then add 4 ml glycol-alkali mixture, mix carefully and read as described in I.

Calculation :

$$\frac{\text{Extinction of sample}}{\text{Extinction of standard}} \times \frac{\text{phenolphthalein}}{\text{in standard } (\mu g)} \times \frac{100}{\text{serum (ml)}} \times \frac{1}{\text{incubation time(hr)}}$$

$$= \text{units of glucuronidase/100 ml serum}$$

One unit beta-glucuronidase is the amount that releases 1 μg phenolphthalein per hour under the given experimental conditions.

RESULTS

Table 1 shows the statistical evaluation of the results. Figures 1～4 gives the results from all experiments made in diagrams.

As already mentioned, the method used in the present investigation was that given by GOLDSTEIN (1961)[9]. The investigator reported the results of analyses made on 6 subjects. Unfortunately, the sex of the subjects not reported, or whether they were normal. A very large spread of readings was found within the individual analysis groups, and a pronounced difference between the results obtained by using the four methods. He thus found: Method I, 877 (593—1034); Method IV, 881 (590—990); Method II, 336 (254—400): Method III, 317 (153—498) units.

A few authors, for example COHEN *et al.* (1951)[3], find that when the analysis is done by method I there is a significant difference between the values

Table 1. Serum beta-glucuronidase activity in normal subjects and in patients suffering from neurological or mental biseases.

	n	s^2	M	t	min.-max.
Group 1					
Normal	26	147167	878		275-1993
Multiple Sclerosis	12	155003	946	0.48	460-1440
Psychoses	7	149858	584	−1.71	152-1069
Neuroses	14	213474	1113	1.76	495-2263
Epilepsy	8	148202	1538	4.05***	799-2068
Group 2					
Normal	25	15456	236		33-415
Multiple Sclerosis	11	7993	231	−0.10	145-461
Psychoses	7	16799	149	−1.54	32-425
Neuroses	14	18036	262	0.60	88-553
Epilepsy	8	37040	391	2.14*	76-612
Group 3					
Normal	26	8440	158		24-779
Multiple Sclerosis	12	4432	113	−1.69	16-286
Psychoses	4	5046	209	1.30	141-292
Neuroses	14	59324	415	3.82**	139-999
Epilepsy	8	63049	434	3.05**	202-991
Group 4					
Normal	25	104219	703		253-1459
Multiple Sclerosis	12	110670	955	1.80	566-1535
Psychoses	7	234805	646	−0.34	135-1464
Neuroses	14	185581	877	1.31	443-1955
Epilepsy	8	305696	1136	2.67**	219-1845

n=number of cases, s^2=variance, M=mean value for activity in units. t=t−test value. One unit of beta-glucuronidase is the activity resulting in the release of 1 μg phenolphthalein per hour under the given experimental conditions. The significant t-values are indicated by *, ** and ***, depending on whether the value exceeds the 5 per cent, 1 per cent or 0.1 per cent probability level.

in men (n-16, mean 926, range 640—1360, standard error 44) and women (n-33, mean 670, range 300—1100, standard error 41). In the material of the present investigation, only a small and statistically insignificant difference was found between the normal men and women examined, so that they are grouped together both in the normal material and in the separate disease groups.

The table and figures show that in the present material there is also very great scattering among the values in each separate group.

The analysis results obtained here show more or less the same variations as

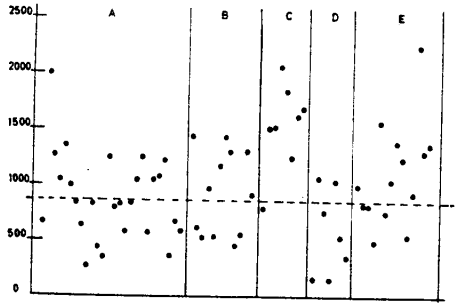

Fig. 1 Serum-glucuronidase in units. Each single point represents one subject examined. A, Controls (men and women); B, multiple sclerosis, (men and women); C, epilepsy (men and women); D, Psychosis or mental disease (women); E, neurosis (men and women). Ordinate: Ordinate: units.

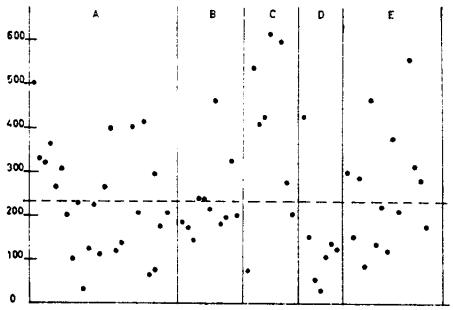

Fig. 2 Text as used in Fig. 1

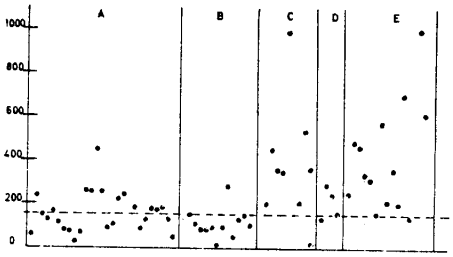

Fig. 3 Text as used in Fig. 1

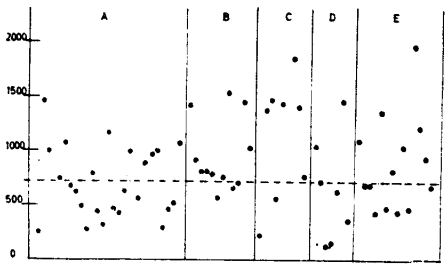

Fig. 4 Text as used in Fig. 1

found by GOLDSTEIN in determining serum beta-glucuronidase activity by various protein-precipitating procedures.

If the values found are evaluated statistically, calculating a mean value and variance for normal subjects and each patient group, and the standard error the difference between the normal group and the patient groups is evaluated by the *t*-test, it will be found that the group representing patients suffering from epilepsy differs significantly from the normal group for all of the four methods used.

The other patient groups do not differ significantly from the normal, with one exception, namely the group of those suffering from neurosis. In this group, use of method No. III. (removal of protein by heat coagulation) shows enzyme activity deviating sinificantly from normal.

These analyses are made on different days so that a systematic error can be excluded, as analyses were made on the same days in the case of other patient groups.

SUMMARY

Using the method given by GOLDSTEIN (1961)[9] for the determination of serum beta-glucuronidase activity, this value was determined in both normal and patients with epilepsy, neuroses, psychoses and multiple sclerosis. Of the patient groups examined, the group of those suffering from epilepsy is the only one showing any difference of statistical significance for all four methods of determination.

The group of patients suffering from neuroses differs significantly from the normal group as regards the results got by the method of heat coagulation for removal of the proteins.

The material is however too small to provide any explanation of the results, but it appears to show that a determination of serum glucuronidase activity may be of interest in groups of diseases other than malignant tumors.

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