A close positive correlation has been found in this laboratory between serum \( \beta \)-glucuronidase activity and blood glucose level immediately following administration of glucose, epinephrine and insulin to normal fasted rabbits (1). Similar correlation has also been shown in hyperglycemia of a long term produced by alloxan administration to rabbits (2). On the other hand, it has been known that serum \( \beta \)-glucuronidase activity is higher in diabetic patients than in normal subjects (3–9). In view of the possible bearing of the serum \( \beta \)-glucuronidase-blood glucose level correlation on diabetes mellitus in man, it was felt essentially important to investigate this relationship in more detail.

There are several questions to be answered in this study: 1) does the serum \( \beta \)-glucuronidase activity increase occur following administration of sugars other than glucose; 2) specifically, is it not merely due to physicochemical changes of blood such as the increase in the osmolarity; 3) if this phenomenon is specific for certain sugars, is such specificity related to possible disturbances in glucose metabolism; and, 4) what effects do different types of antidiabetics have on serum \( \beta \)-glucuronidase activity.

The study reported here thus describes the relationships between the serum \( \beta \)-glucuronidase activity and blood glucose level and between the activity and the levels of administered sugars, following administration of various sugars and antidiabetics to rabbits.

**MATERIALS AND METHODS**

**Experimental animals**

Healthy male white rabbits bred in our laboratories, weighing 2.5 to 3.5 kg, were used. Animals were individually caged, fed with RC-5 (Oriental Yeast Co., Ltd., Tokyo) and allowed to drink water *ad libitum*.

**Chemicals**

Tolbutamide (1-butyl-3-p-tolylsulfonyl urea) was obtained from Chugai Pharmaceutical Co., Ltd. Carbutamide (1-butyl-3-sulfanilyl urea) and phenformin (1-phenethylbiguanide) were obtained from Ono Pharmaceutical Co., Ltd. Blood Sugar-Test for the determination of blood glucose level was purchased from C.P. Boehringer & Soehene.
GmbH, Mannheim, Germany. All other chemicals including sugars were obtained from commercial sources.

Dosing and blood sample collection

Twenty-four hours after the last feeding, various sugars or antidiabetics as a 20 to 40% solution in 0.15 M NaCl were administered intravenously or orally to the animals. All blood samples were drawn from the ear vein before and at proper intervals after the administration.

Assay method for serum β-glucuronidase activity

Serum β-glucuronidase activity was assayed with p-nitrophenyl β-D-glucuronide as the substrate at pH 4.5 in 0.1 M acetate buffer (1). Enzyme activity was expressed as µg of p-nitrophenol released per hour per dl of serum.

Determinations of blood sugar levels

Blood samples were deproteinized by adding 0.33 M perchloric acid, centrifuged for 10 minutes at 3,000 r.p.m., and glucose concentration in the supernatant was determined by a glucose oxidase method employing the Blood Sugar-Test. Similarly, total sugars in the supernatant were determined by anthron method for hexoses (10), pentoses (11) and 2-desoxy-glucose (12). The difference between the glucose and total sugar concentration was considered as the blood level of administered sugar. Glucosamine in the supernatant was determined by the method of Gardell (13).

RESULTS

β-Glucuronidase activity in serum was measured following intravenous administration of hexoses (glucose, mannose, fructose and galactose), glucose derivatives (glucosamine, N-acetylglucosamine and 2-desoxyglucose), pentoses (ribose and xylose) and a disaccharide (lactose) to fasted rabbits. Concomitant measurement of the blood concentration of

| Treatment                  | Percentage maximal increment in β-glucuronidase activity | Time of peak β-glucuronidase activity | Percentage maximal increment in blood glucose level |
|----------------------------|----------------------------------------------------------|--------------------------------------|---------------------------------------------------|
| Glucose (1.0 g/kg, i.v.)   | 40, 47, 57, 79                                           | 0.5                                  | 260, 230, 281, 310                                |
| Fructose (1.0 g/kg, i.v.)  | 102, 153, 199, 289                                        | 0.5                                  | 49, 33, 32, 63                                   |
| Mannose (1.0 g/kg, i.v.)   | 149, 175, 213, 239                                       | 1.0                                  | 35, 47, 37, 49                                  |
| Glucosamine (500 mg/kg, i.v.) | 85, 96, 112                                           | 1.0                                  | 40, 41, 73                                       |
| N-Acetylglucosamine (500 mg/kg, i.v.) | 50, 79                      | 0.5                                  | 54, 44                                           |
| 2-Desoxyglucose (500 mg/kg, i.v.) | 72, 84                      | 3.0                                  | 138, 113                                         |
| Galactose (1.0 g/kg, i.v.) | 4, 13, 21                                               |                                      | 1, 13, 32                                       |
| Ribose (1.0 g/kg, i.v.)    | 5, 9                                                      |                                      | 11, 12                                           |
| Xylose (1.0 g/kg, i.v.)    | 12, 14                                                   |                                      | 12, 13                                           |
| Lactose (1.0 g/kg, i.v.)   | 22, 25                                                   |                                      | 5, 8                                              |

Respective data from individual animals listed in the presented order.

* Identical time of peak β-glucuronidase activity following any set of treatment.
glucose in all experiments and of administered sugars in most of the experiments were also made.

A summary of the results obtained in this study is given in Table 1.

The representative time courses of β-glucuronidase activity as well as the levels of glucose and/or administered sugars are illustrated in Fig. 1 for the six sugars, administration of which produced a marked increase in serum β-glucuronidase activity. The elevation of the enzyme activity was immediate with glucose, fructose and N-acetylglucosamine, and relatively rapid with mannose and glucosamine while it increased gradually with 2-desoxyglucose. When the same size of dose (1.0 g/kg) was administered intravenously, fructose and mannose yielded more than 2.0 fold increases while glucose produced a maximal increase of only 40 to 80% in the β-glucuronidase activity (Table 1). The other sugars

![Fig. 1. Changes in blood glucose levels, serum β-glucuronidase activities and administered sugar levels in rabbits.](image-url)

Keys: ·····, serum β-glucuronidase activity.
- - - - , blood glucose level.
--- , administered sugar level.
were given at a lower dose level (500 mg/kg). It was however noted that this lower dose of glucosamine, N-acetylglucosamine and 2-desoxyglucose increased the serum \(\beta\)-glucuronidase activity to the same extent as, or more than did the doubled dose of glucose. With mannose and 2-desoxyglucose, \(\beta\)-glucuronidase disappeared from serum more slowly than with the other sugars; in the latter case, it declined nearly to the preadministration value within 3 hours after the peak activity. Although the administration of 2-desoxyglucose only among other sugars produced a marked increase of the blood glucose level which was parallel in time course to that of serum \(\beta\)-glucuronidase activity over a long period, there were observed more or less increases in blood glucose level when other \(\beta\)-glucuronidase elevating sugars were administered (except mannose). Generally, the time course of \(\beta\)-glucuronidase change appeared to be unrelated to that of the sugars administered except in the case of glucose. Thus, the elimination of fructose, mannose, 2-desoxyglucose and glucosamine from blood occurred much more rapidly than the increase and decline of serum \(\beta\)-glucuronidase activity.

The results following intravenous administration of 1.0 g/kg of sugars which produced no or relatively minor changes in serum \(\beta\)-glucuronidase activity are shown in Fig. 2. These sugars include galactose, ribose, xylose and lactose. There was no appreciable change in blood glucose level with galactose, ribose and lactose while, with xylose, a slight but consistent elevation was noted in the \(\beta\)-glucuronidase activity accompanied in one

![Fig. 2](image-url)

*Fig. 2.* Changes in blood glucose levels, serum \(\beta\)-glucuronidase activities and administered sugar levels in rabbits.

*Keys:* ---, serum \(\beta\)-glucuronidase activity.
---, blood glucose level.
---, administered sugar level.
instance by a gradual increase in blood glucose level.

The effects of different types of antidiabetics on serum β-glucuronidase activity and blood glucose level are shown in Fig. 3. The administration of two sulfonylureas, i.e. tolbutamide (p.o.) and carbutamide (i.v.), resulted in a decrease of serum β-glucuronidase activity which was closely related to the decrease in blood glucose level. On the other hand, the β-glucuronidase activity rather increased following oral administration of phenformin, indicating an apparent discrepancy between serum β-glucuronidase activity and blood glucose level.

DISCUSSION

It is evident that the administration of certain sugars other than glucose induces an elevation of serum β-glucuronidase activity in rabbits. It is also evident that the increase in the serum β-glucuronidase activity is not due to simple physicochemical changes of the blood such as osmolarity increase since not all sugars tested produce the elevation of the enzyme activity in serum. Particularly, the failure of galactose to exert such an effect points out the fact that to be a hexose is not sufficient for a sugar to be a serum β-glucuronidase activity elevator. This property is thus specific for glucose, fructose, mannose, 2-desoxyglucose and glucosamine among the sugars tested in this study. There is however a difference between glucose and other sugars in terms of the relationship between the increase in serum β-glucuronidase activity and the blood levels of administered sugars. While glucose administration resulted in an immediate increase and a decline of serum β-glucuronidase activity which was paralleled closely by the blood glucose levels, the responses of the serum enzyme following administration of the other sugars were somewhat delayed (Fig. 1). The results of the study using sulfonylureas (Fig. 3) and 2-desoxyglucose also show the particularly close relationship between blood glucose levels and serum β-glucuronidase activity, in addition to those obtained in the previous study using insulin and epine-
phrine (1). It was further pointed out in RESULTS that the administration of a serum \( \beta \)-
glucuronidase activity elevating sugar yielded a more or less increase in the blood glucose
levels while there was no appreciable change in the latter following administration of
sugars which did not affect serum \( \beta \)-glucuronidase (Fig. 2). These observations, although
indirectly, suggests as one of possibilities that the disturbance in certain process for the
transport, distribution and/or metabolism of glucose is related to the serum \( \beta \)-glucuronidase
elevation. This disturbance may well be reflected in glucose levels in blood in the case of
2-desoxyglucose or may not in the case of other sugars.

The next logical step is to characterize the locus of the possible disturbance. Table
2 lists the known effects of the sugars used in this study on the insulin secretion (14) and
on the glucokinase (15) as well as the steric configuration of these sugars. It is readily
apparent that there is no resemblance between the effects on insulin secretion and on the
serum \( \beta \)-glucuronidase activity of respective sugars. The insulin secretion is known to
be stimulated by glucose, fructose and mannose but rather inhibited by glucosamine and
2-desoxyglucose (14). On the other hand, it was shown in this study that the intravenous
administration of these sugars increases serum \( \beta \)-glucuronidase activity without exception.
Ribose does not affect the serum enzyme activity but stimulates the insulin secretion (14).
It is unlikely therefore that the serum \( \beta \)-glucuronidase activity elevation following the sugar
administration is either mediated by mechanism similar to insulin release or a result of
inhibition of insulin secretion.

It then becomes significant to find out a parallelism between the inhibitory effect on
the glucokinase of rabbit liver (15) and serum \( \beta \)-glucuronidase elevation effect of sugars
(the third column in Table 2). The glucokinase, which is an enzyme localized predomi-
nantly in liver and have a high Km value for glucose (16–18), catalyzes the phosphorylation
of glucose to glucose-6-phosphate. This enzyme is said to be responsible for glycogen

| Sugars            | Serum \( \beta \)-glucuronidase activity* | Insulin secretion* | Ki values for glucokinase (mM) | Modified with respect to glucose at |
|-------------------|------------------------------------------|--------------------|---------------------------------|-------------------------------------|
| Glucose           | ↑                                        | ↑                  | --                              | --                                  |
| Mannose           | ↑                                        | ↑                  | 16                              | C-1, C-2                           |
| Fructose          | ↑                                        | ↑                  | **                              | C-2                                 |
| Glucosamine       | ↑                                        | ↓                  | 1                               | C-2                                 |
| N-Acetylglucosamine | ↑                                       | **                 | 0.3                             | C-2                                 |
| 2-Desoxyglucose   | ↑                                        | ↓                  | 23                              | C-2                                 |
| Galactose         | →                                        | **                 | 200                             | C-4                                 |
| Xylose            | →                                        | **                 | 130                             | C-6                                 |
| Ribose            | →                                        | ↑                  | **                              | C-3, C-6                            |
| Lactose           | →                                        | **                 | **                              | Disaccaride                         |

* ↑: Increased  ↓: Decreased  →: Unchanged
**Data not available.
synthesis in liver by subsequent steps (16). As judged from the Ki values (the inhibition is competitive), mannose, glucosamine, N-acetylglucosamine and 2-desoxyglucose are all relatively strong inhibitors of this enzyme and all elevate serum β-glucuronidase activity, while galactose and xylose which are relatively weak inhibitors have no or an only minor effect on the serum enzyme activity. A complete parallelism between the degrees of β-glucuronidase elevation and glucokinase inhibition cannot be expected since the latter are the values determined in vitro. The in vivo inhibition of glucokinase would result in hepatocellular accumulation of free glucose, and consequently, may in certain instances reflected in a delayed increase in the blood glucose levels. Although insulin is known to induce glucokinase synthesis (19), the decrease of serum β-glucuronidase activity following insulin administration reported previously (1) is too immediate to rely on such a mechanism. This effect is rather secondary to the decrease in blood glucose levels induced by insulin. In this connection, however, it is interesting to note that the glucokinase disappears from the liver of alloxan-diabetic rats (20), and that the serum β-glucuronidase activity increases in alloxan-diabetic rabbits (2). This is again in support of the hypothesis that the hepatocellular glucose accumulation leads to an elevation of serum β-glucuronidase activity. While the effect of 2-desoxyglucose may also be through its known stimulating action on epinephrine secretion (21), this either does not discourage the present hypothesis.

It should be recognized, however, that it is impossible to formulate a unitary mechanism by which all the changes in serum β-glucuronidase activity can be interpreted. The effect of fructose is difficult to explain on the basis of glucokinase inhibition since its Ki value for liver glucokinase of rats (although not of rabbits as used in this study) has been reported to be considerably high (22). Difficulties are also encountered in interpreting the apparent discrepancies between the serum β-glucuronidase activity and blood glucose levels following administration of phenformin observed in this study (Fig. 3) and of hydrocortisone in the previous study (2). In this respect, it requires particular attention that insulin-dependent changes in blood glucose levels are always accompanied by parallel changes in serum β-glucuronidase activity, as evidenced by the effects of the administrations of insulin (1), alloxan (2) and sulfonyluraes (Fig. 3).

Regardless of what the mechanism(s) responsible for the serum β-glucuronidase increase may be, considerations on the steric configurations of the sugars afford an important generalization. Structurally, as listed in Table 2 (the fourth column), the sugars which can produce a serum β-glucuronidase elevation are different from glucose only at C-2 positions, while the other sugars are different at C-3 and C-4 positions, lack C-6, or a disaccharide. Fructose which is a ketose and differs also at C-1 position represents an exception in accord with its exceptionally high Ki value for rat liver glucokinase (22). It may be that the presence of a reducing group is necessary for serum β-glucuronidase increase since an additional experiment shows that mannitol, being different from glucose at both C-1 and C-2 positions, is ineffective in elevating the serum enzyme activity.
SERUM $\beta$-GLUCURONIDASE ACTIVITY

SUMMARY

Studies on the serum $\beta$-glucuronidase activity following the administration of various sugars and antidiabetics to rabbits were carried out with the following results.

Serum $\beta$-glucuronidase activity was increased significantly after the intravenous administration of fructose, mannose, glucosamine, N-acetylglucosamine and 2-desoxyglucose in addition to that of glucose described previously while it remained nearly unaltered after the intravenous administration of galactose, ribose, xylose and lactose. The possible link of these effects with glucose metabolism was discussed in terms of glucokinase inhibition and steric configuration of sugars. Serum $\beta$-glucuronidase activity was decreased after the administration of sulfonylureas (tolbutamide and carbutamide) in parallel to the decrease in blood glucose levels while it was increased opposingly to decreased blood glucose levels, after the administration of a different type of antidiabetic, phenformin. This finding is another evidence to indicate that the insulin-dependent changes in blood glucose levels are invariably accompanied by parallel changes in serum $\beta$-glucuronidase activity.

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