TECHNICAL PAPERS

B-Glucuronidase Activity of the Blood and Tissues of Obstetrical and Surgical Patients[1]

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The enzyme β-glucuronidase hydrolyzes a large variety of conjugated glucuronides. Its function *in vivo* is believed to be the catalysis of glucuronide conjugation (*1*). In previous work, its relation to the metabolism of the estrogens has been demonstrated (*4*). In humans, glucuronides of the estrogens and their derivatives are excreted in the urine. The process of glucuronide formation should not be regarded necessarily as a detoxication mechanism but as one of "metabolic conjugation" (*2*).

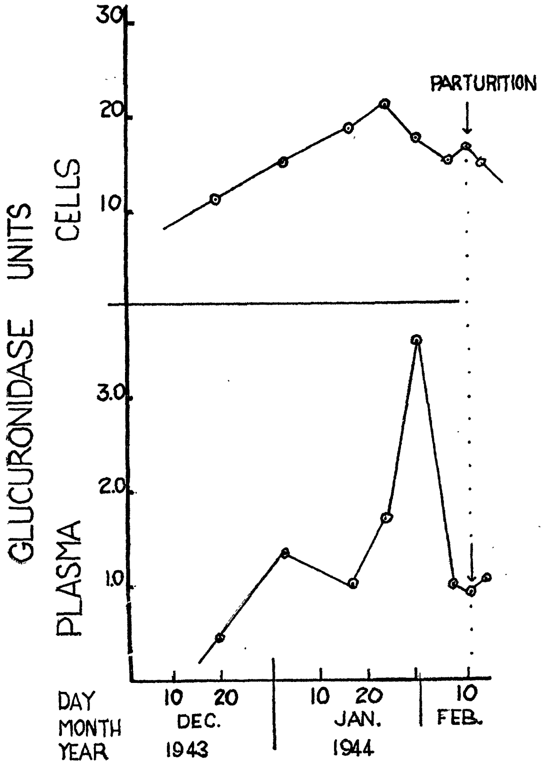

Fig. 1. Alterations in the β-glucuronidase activity of the blood of one patient as related to the last trimester of pregnancy and parturition. A glucuronidase unit here is defined as 1 mg. of glucuronic acid liberated from menthol glucuronide per hour at 38° per 100 cc. volume under standard conditions (*1, 2*).

Conjugated estrogens are excreted in large amounts during pregnancy. Since β-glucuronidase appears to be involved in the conjugation of the steroid hormones, its activity was studied in the blood cells and plasma in the various stages of human pregnancy. The following observations were made on the 30 women studied.[2]

As in most humans, there is a much greater concentration of the enzyme in the blood cells as compared with the plasma. However, the enzyme activity in the cells increases as pregnancy progresses and remains elevated for a short period after parturition. In the plasma, the enzyme shows peaks of increased activity and usually drops to normal levels after parturition. An example of the alterations in blood glucuronidase with pregnancy is shown in Fig. 1. While this study was in progress, it was noted that in one case there was a high plasma glucuronidase associated with early toxemia. These preliminary observations suggest that the study of the blood glucuronidase in a large number of women may lead to a better understanding of the process of gestation and may also yield data of diagnostic value.

TABLE 1*

Patient	Pathological diagnosis		Units† of glucuronidase activity			
			Tissue		Blood	
	Lesion	Organ	Tumor	Normal	Cells	Plasma
M.	Carcinoma	Breast	900	94	260	145
R.	"	"	1,930	62	579	118
K.	Fibroadenoma	"	393	325	563	150
K.	Carcinoma	Esophagus	1,510	750	1,098	278
B.	"	"	2,160	650	399	187
L.	"	Stomach	3,180	940	1,155	236
V.	"	Colon	900	1,930	200	266
O.	"	"	1,360	1,760	448	228
M.	"	"	5,250	2,490	633	91

* These data are taken from work in progress, which is being done in collaboration with A. J. Anlyan. The full publication will appear at a later date.

† One unit is defined as 1 μg. of phenolphthalein liberated from phenolphthalein mono β-glucuronide per hour at 38° from 1 gram of moist tissue or from a 100-cc. volume of cells or plasma under standard conditions (*3,5*).

Since mammary tumors have been induced in animals by the prolonged administration of estrogens, it seemed desirable to study the β-glucuronidase activity of various tumor tissues obtained at operation. Wherever possible, the corresponding uninvolved tissue was also assayed. Plasma and blood cell glucuronidase were determined before operation. Some of the data are given in Table 1.

It is clear that in carcinoma of the breast the tumor contains from 10 to 20 times as much enzyme as the uninvolved

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[2] The cooperation of Richard Forman is gratefully acknowledged. These patients were studied with the permission of the Department of Obstetrics and Gynecology, Bowman Gray School of Medicine, Winston-Salem, North Carolina.

breast tissue. No difference from normal was noted in the case of the benign fibroadenoma. In carcinoma of the gastrointestinal tract an elevated concentration of the enzyme is apparent in 4 of 6 patients.

From these few cases there seems to be no correlation of the blood glucuronidase with the incidence of cancer. However, one may speculate as to the reason for the relatively high glucuronidase activity in mammary tumors. Since glucuronidase activity has been found associated with the action of the estrogenic hormones (*4*), this suggests the accumulation of significant amounts of sex hormone in the mammary tumor. In cancer of the gastrointestinal tract, the question arises as to whether or not excessive amounts of estrogen may be present, in view of the elevated glucuronidase content.

References

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Relabeling of the Cis and Trans Isomers of 1,3-Dimethylcyclohexane

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Evidence pointing to the necessity of relabeling the cis and trans isomers of 1,3-dimethylcyclohexane has been recently presented (*6*). The pertinent experimental facts may be summarized as follows: (a) The 1,3-dimethylcyclohexanes were synthesized by Skita and Schneck (*7*), who prepared a mixture of the cis and trans isomers by hydrogenating m-xylene, and who identified for the first time the lower-boiling and higher-boiling isomers as the trans and cis isomers, respectively, according to von Auwers' rule (*8*), and analogous to the isomers of 1,2-dimethylcyclohexane and 1,4-dimethylcyclohexane. (For these isomers, the lower-boiling one also has a lower refractive index and lower density.) (b) The low-temperature measurements of Oliver, Todd, and Huffman (*6*), coupled with values of the standard entropies of vaporization (*2*), yield experimental values for the standard entropy in the gaseous state at 25°C. such that, for the cis and trans isomers, the lower-boiling isomer has a standard entropy lower than that of the higher-boiling isomer by about 1–1.5 cal./deg. mole, in each of the three pairs, 1,2-dimethylcyclohexane, 1,3-dimethylcyclohexane, and 1,4-dimethylcyclohexane. (c) The energies of isomerization reported by Prosen, Johnson, and Rossini (*6*) for the gas state at 0° K are such that the three lower-boiling isomers have an energy content near 2 kcal./mole less than that of the corresponding higher-boiling isomer. (d) The data (*3*) giving the relative amounts of the cis and trans isomers formed in the hydrogenation (in the range 200°–250°C.) of each of the three xylenes show that the lower-boiling isomer is in each case the more abundant, the relative amounts being in accord, within the combined limits of uncertainty, with the values calculated (*4*) thermodynamically from the experimental entropies and heats of isomerization. The foregoing experimental facts are consistent in indicating a lower energy content, lower entropy, lower density, and lower refractive index for the lower-boiling isomer of the cis- and trans-1,2-, 1,3-, and 1,4-dimethylcyclohexanes.

It is also a matter of experimental fact that, among pairs of geometric isomers, the one possessing less steric hindrance has the lower energy content and usually has the lower boiling point, lower density, and lower refractive index. Various complex situations give exceptions to this rule. If the molecular configuration is such as to produce less steric hindrance in the trans isomer, the latter will normally have the lower boiling point, lower energy, etc., and the cis isomer will normally have the higher boiling point, higher energy, etc. This is the situation in the monoolefins (such as cis- and trans-2-butene) and in the cis- and trans-1,2-dimethylcyclopentanes, in which cases the trans isomer is the lower-boiling, etc.[1] In these cases, the name trans is synonymous with less steric hindrance.

But in the case of the cis and trans isomers of the 1,2-, and 1,3-, and the 1,4-dimethylcyclohexanes, Pitzer and Beckett (*6*) have shown that the trans configuration is not uniformly associated with the isomer of less steric hindrance. As has already been explained in detail by Pitzer, Beckett, and Spitzer (*6*), substantially all the molecules of cyclohexane are in the "chair" or symmetrically staggered configuration in which the successive CH_2 groups comprising the ring are alternately twisted up and down from a plane passing through the ring of carbon atoms. The result is that 6 of the hydrogen atoms lie in an equatorial belt extending out from the ring of carbon atoms, while the remaining hydrogen atoms are perpendicular to the plane through the ring of carbon atoms, three being above and called north polar, and three being below and called south polar. Of the 6 hydrogen atoms which are "cis" to one another, three may be described as north polar and three as equatorial. Of the two hydrogen atoms on each carbon atom, one is polar and one equatorial. If the 6 carbon atoms are all twisted through a single plane over to the opposite chair configuration, all hydrogen atoms originally polar become equatorial and vice versa. With this situation, there thus exists among the 1,2-, the 1,3-, and the 1,4-dimethylcyclohexanes two tautomeric forms for each isomer. Considering only the tautomeric form of lower energy in each case where the energies are different, the trans-1,2-, the cis-1,3-, and the trans-1,4-dimethylcyclohexanes each have both methyl groups equatorial, while the cis-1,2, the trans-1,3, and the cis-1,4-dimethylcyclohexanes each have one methyl group equatorial and one polar. Changing one methyl group from an equatorial to a polar position leads to an increase in the steric hindrance in the molecule and a consequent increase in its energy.

The statistical calculations (*6*) lead to values of the standard entropy lower for trans-1,2-, cis-1,3-, and trans-1,4-, respectively, than for cis-1,2-, trans-1,3-, and cis-1,4-dimethylcyclohexane. The analysis of the molecular structure also indicates that, in each pair (cis and trans), the isomers of lower energy should be trans-1,2-, cis-1,3-, and trans-1,4-dimethylcyclohexane. Furthermore, the calculated values of these differences agree quantitatively with the experimental

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[1] See, however, G. Chiurdoglu (*Bull. Soc. Chim. Belg.*, 1944, 53, 45), who has concluded, on the basis of rates of reaction, that in the 1,2-dimethylcyclopentanes, the cis isomer is the lower-boiling. We are not inclined to regard this type of evidence as at all conclusive.