Reports

Enzymatic Syntheses of Oligo- and Polysaccharides Containing D-Glucosamine

The synthesis of several oligosaccharides by carbohydrases has now been firmly established (*1–3*). Recently, Hestrin *et al.* (*4*) have demonstrated the formation of numerous glycosides by the action of levansucrase (from *Aerobacter levanicum*) on sucrose. Oligosaccharides, containing N-acetylglucosamine, are also reported to be formed by the action of *Lactobacillus bifidus* and preparations of mammalian tissue on lactose and N-acetylglucosamine (*5*). We have now shown that amino sugars act as acceptors in the transglycosylation reactions yielding oligo- and polysaccharides. This report (*6*) is a preliminary account of our observations (*7*).

By use of the method of estimation of glucose by notatin (*8*), it was found that D-glucosamine inhibits competitively the hydrolysis of sucrose by yeast invertase. The amino sugar, however, was found to have no inhibitory effect on the rate of synthesis of oligosaccharides; in fact, a slight stimulatory effect was observed. Further evidence was obtained by incubating invertase with a mixture of sucrose and glucose-U-C^{14}. The products were separated by paper chromatography, and their radioactivities were measured. When D-glucosamine was added to the mixture of sucrose, glucose-U-C^{14}, and invertase, larger quantities of labeled sucrose and oligosaccharides were formed (see *9*).

Aliquots of a mixture containing yeast invertase, sucrose-U-C^{14}, and D-glucosamine, after incubation at 25°C for varying periods of time (10 to 60 minutes) were subjected to electrophoresis on paper in 0.2*N* acetic acid. The filter paper strips were sprayed with ninhydrin. Two spots besides the one due to D-glucosamine were detected. The fructose content of these two spots was revealed by spraying them with the ketose-specific urea-HCl reagent. It was inferred from the relative mobilities of these amino sugar-oligosaccharides that one of them was a disaccharide and the other a trisaccharide.

The action of yeast lactase ("lactase-B") (*10*) on *o*-nitrophenyl-β-D-galactopyranoside was found to be inhibited by D-glucosamine, N-acetylglucosamine, and D-galactosamine. D-Galactosamine inhibits competitively, whereas D-glucosamine and N-acetylglucosamine inhibit noncompetitively.

Experiments in which galactose-U-C^{14} or glucose-U-C^{14} was used showed that either D-glucosamine or N-acetylglucosamine stimulates the synthesis of radioactive oligosaccharides and radioactive lactose formed by the action of lactase-B on a mixture of lactose and galactose-U-C^{14} or glucose-U-C^{14}. By electrophoretic and chromatographic techniques, four oligosaccharide components containing D-glucosamine and two oligosaccharide components containing N-acetylglucosamine were detected (see *2, 5, 11*).

Synthesis of levan from sucrose takes place in the presence of a cell-free extract of *Aerobacter aerogenes* grown adaptively on sucrose (see *4*). While several oligosaccharides are also formed, we have been unable to detect the formation of any significant quantity of free fructose or free glucose from sucrose. The presence of D-glucosamine or N-acetylglucosamine diminishes the amount of fructose in the isolated polysaccharide (see *12*). The polysaccharide synthesized by *Aerobacter aerogenes,* in the presence of sucrose and glucose, was hydrolyzed with 0.5 percent oxalic acid and was found to contain glucose as well as fructose. The polysaccharides synthesized by *Aerobacter aerogenes* in the presence of sucrose and D-glucosamine or N-acetylglucosamine were found to contain fructose, the respective amino sugar, and small amounts of glucose. Further evidence for the entry of the amino sugar into the polysaccharide was obtained by employing sucrose-U-C^{14} as substrate and estimating the specific activities of the labeled polysaccharides formed. The specific radioactivities of the polysaccharides synthesized in the presence of the amino sugars were found to be less than those of the polysaccharides synthesized in the absence of the amino sugars. Levansucrase from *Aerobacter aerogenes* was found to catalyze the formation of at least five detectable oligosaccharides containing glucosamine from a mixture of sucrose and D-glucosamine (Table 1).

Whenever D-glucosamine or N-acetylglucosamine was present in the reaction mixture containing *Aerobacter aerogenes* extract and sucrose, a stimulation of the rate of formation of free fructose and glucose, as well as of that of oligosaccharides and polysaccharide, was observed.

We have shown that, in the presence of yeast invertase or levan sucrase from *Aerobacter aerogenes,* several amino sugar-oligosaccharides are synthesized from sucrose and D-glucosamine. Moreover, polysaccharide containing D-glucosamine is formed by an extract of *Aerobacter aerogenes* from sucrose and D-glucosamine. Oligosaccharides containing D-glucosamine or N-acetylglucosamine are also formed by the action of yeast lactase on a mixture of lactase and amino sugar. It is therefore evident that the formation of heterogeneous mucopolysaccharide may be brought about by the transglycosylation mechanism. Such formation of oligo- and polysaccharides containing D-glucosamine may be studied quantitatively by the use of labeled sugars and electrophoretic separation. The fact that the amino

Table 1. Formation of oligosaccharides containing D-glucosamine by an extract of *Aerobacter aerogenes* in the presence of sucrose-U-C^{14} (0.18*M*) and D-glucosamine (0.3*M*). The reaction mixture contained *A. aerogenes* extract (0.1 ml), sucrose-U-C^{14} (total activity about 1 μc), citrate-phosphate buffer at *p*H 5.4 (0.1 ml), and D-glucosamine (brought to *p*H 5.4). The total volume was 0.5 ml. The mixture was incubated at 37°C under toluene. Ten-microliter aliquots were spotted on Whatman 3 MM paper. Electrophoresis was carried out at 500 v, 5 to 10 ma, for 3 hours in acetic acid buffer (0.2*N*).

Fraction after electrophoresis	Activity of fractions from 10 μl spot (count/min)	
	Incubation 60 min	Incubation 120 min
Origin	2946	2730
Highest polymer: III	217	331
II_I	52	139
II	70	200
I (probably disaccharide)	35	52

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sugars inhibit the hydrolytic activities of yeast invertase and yeast lactase, but not their synthetic activities, may point to the existence of two separate enzymes or of two different glycosyl-enzyme intermediates, one concerned with the hydrolysis of the substrates into their monosaccharide constituents, the other with the synthesis of oligosaccharides.

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References and Notes

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Increased Radioresistance through Heterosis

Strain differences in radiosensitivity of mice have been recognized for some time (*1–4*) even when the variables of age (*3, 5*), sex (*2, 6*), and dose rate (*7*), have been eliminated or controlled. These strain differences have not been explained, except that Brecker and Cronkite (*8*) tried to correlate the degree of injury to bone marrow with species differences.

Inbreeding has long been recognized as a condition leading to reduction in vigor of the species; the opposite practice—namely, outcrossing between fertile strains—has often resulted in first-generation hybrids which are, in certain respects, more vigorous than either of the parental stocks. This is known in genetics as "heterosis." When two divergent strains are crossed, only the first generation resulting therefrom tends to show the maximum beneficial effects in terms of size and vitality. When two diverse groups of heritable factors are brought together, homozygosity is reduced, and the more frequently deleterious recessive characters will be temporarily masked. The individual thus tends to exhibit vitality exceeding the vitality of either of the parent stocks.

Realizing that there are species and strain differences in radiosensitivity (or its reciprocal, radioresistance) and that even closely related and readily interbred mice do not necessarily have the same $LD_{50/30}$ value, we devised an experiment (*9*) to determine whether there might be any heterotic effect in hybrids between the CF_1 and C_{57} black/6 strains of mice. These two strains were inbred randomly for many generations prior to the production of the animals used in this experiment. At the same time a cross was made between C_{57} males and CF_1 females to produce the hybrid strain. The cross was made in this direction because it was found that C_{57} males were somewhat more aggressive than the CF_1 males, and mating resulted more frequently. It is not believed that the opposite cross would have produced F_1 genotypes which were any different.

A total of 485 mice were used in this experiment, divided into six categories of strain and sex, with a minimum of 64 mice in the smallest group and a maximum of 107 in the largest.

The radiation facilities used consisted of a Westinghouse Quadrocondex constant potential therapy x-ray machine at 210 kv (peak) and 15 ma, with 0.28-mm Cu and 0.50-mm Al filters, and at a distance of 40 cm from the target to the center of the body of the mouse. Seven mice were x-irradiated simultaneously in a plastic box in which they were free to move within the uniform field of exposure. The dose rate was 152.7 r/min.

It had previously been found that the $LD_{50/30}$ for the C_{57} black mice was a bit higher than that for the CF_1 white mice, and it has been known for some time that the males are more radiosensitive than are the females of the CF_1 strain (*2*). In order that the physical factors of x-irradiation might be the same for all mice of the same sex, it was decided to x-ray the males to 525 r and the females to 600 r when they were 2 months of age. It was hoped that this would give no 0 percent or 100 percent values in mortality and that all of the data would fall between these extremes. The data are all presented in Table 1.

Species differences in radiosensitivity, expressed in different $LD_{50/30}$ values, have never been explained. There is certainly no correlation with the phylogenetic or evolutionary relationships. Why, for instance, should the guinea pig be so sensitive, the hamster more resistant than man, and the chicken about twice as resistant as man? Nevertheless, the radiation-lethality data are highly reproducible and reliable and are used in radiobiological research for the testing of the effects of the so-called protective drugs and of numerous environmental variables.

Many of the factors which condition or alter radiosensitivity are known. Several of these are age, sex, metabolic or physical activity, or both, amount of oxygen in the environment, presence of infection, previous exposure to insult, and presence of certain so-called protective drugs. When all of these variables are equalized for the animals to be tested, we still find that the C_{57} black mice are more resistant to x-irradiation death than are the CF_1 white, even though they are so closely related that they can be freely interbred. The F_1 generation of a cross between these two strains is identical with the black parent in coat color, and yet the mice manifest survival values even better than that of either parent strain. This implies that the sum total of the effect of a large group of dominant characters coming from both parent strains to the heterozygous hybrids must give to these mice an improved radiotolerance. It would be impossible at this stage to point out those dominant characters which provide the increased radioresistance, or even the possible synergism involved. However, the data do substantiate the presumption that recessive mutants tend to be deleterious.

When one examines these data statistically (*10*) the increase in the female mice is seen to exceed the 5 percent level of probability, indicating that the hybrid female is very probably more radioresistant than either of its pure parent stocks. On the other hand, the relatively small increase in the male hybrids is not statistically significant (see Table 1). This might appear to weaken the thesis

Table 1. Heterosis (hybrid vigor) and radiosensitivity. F_1 mice were tested at 2 months of age. The numbers in parentheses represent the number of animals of the category which were irradiated; C_{57} mice are black, CF_1 mice are white, and the hybrids are black (dominant); χ^2 was determined between the hybrid and the C_{57} pure stocks which have the greater resistance.

Mice and radiation dose	Percentage survival				
	Pure	Pure	Hybrid	χ^2	P
F_1 generation	$C_{57} \times C_{57}$	$CF_1 \times CF_1$	$C_{57} \times CF_1$		
Females (+600 r)	20.8 (77)	8.6 (72)	36.5 (96)	5.055	< 0.05
Males (+525 r)	70.3 (64)	33.3 (69)	80.4 (107)	2.262	> 0.10