removed from the data. The remaining population of waveforms falls mainly into one peak, as shown in the third stage. Those waveforms that fall to the left of the arrow are labeled type 2. The residue to the right of the arrow represents various anomalous waveforms arising when two different action potentials almost coincide in time.

A print-out for monitoring the final sorting of the data is shown in Fig. 2; each action potential was labeled by the computer. Errors in classification generally run well under 5 percent and the entire procedure takes several minutes.

Once the action potentials have been sorted, it is easy to examine the statistical properties of the firing patterns of each neuron in the recording as well as the various conditional firing probabilities which can be used for examining interactions between different neurons. A current study of the dorsal cochlear nucleus by these means shows that contiguous neurons interact in various ways that depend partly on the parameters of the stimulus.

G. L. GERSTEIN
W. A. CLARK
Center for Computer Technology and Research in the Biomedical Sciences, Massachusetts Institute of Technology, Cambridge

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9. We thank the staffs of the Research Laboratory of Electronics, M.I.T., and of the TX-2 computer at Lincoln Laboratory, M.I.T., for their technical assistance. This research was supported in part by U.S. Army Signal Corps, the Air Force Office of Scientific Research, and the Office of Naval Research; the National Institutes of Health (grants FR-00142-01 and MH 04737-03); and the National Science Foundation (grant G-16526).

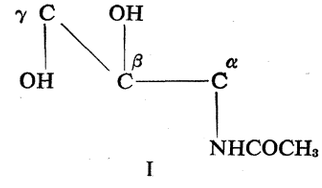
3 January 1964

Conglutination: Specific Inhibition by Carbohydrates

Abstract. *Conglutination of antigen-antibody-complement complexes is inhibited by a number of acetamido sugars, the most efficient being* N-*acetyl*-D-*glucosamine and closely related compounds. The effects of structural modification on activity of* N-*acetyl*-D-*glucosamine are described.*

Chromatographically purified bovine conglutinin (*1*) clumps, or conglutinates, complexes of antigen, antibody, and complement. It has been suggested that this conglutination reaction is due to combination of conglutinin with sites on a component or components of complement which are only exposed after binding to the antigen-antibody aggregate (*2*). To elucidate the chemical basis of specificity of the conglutinin system we have examined a variety of carbohydrates for possible inhibitory activity.

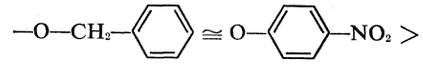
The results (Table 1) suggest that a molecule containing an acetamido sugar plays an important role in determining specificity of the conglutination reaction. A feature of all inhibitory acetamido sugars is the sequence



I

In the case of 3-acetamido-3-deoxy-D-galactose, 3-acetamido-3-deoxy-D-manno-D-galaheptose, and 3-acetamido-3-deoxy-D-glucose, all weak inhibitors, the γ carbon is the anomeric carbon. α-Glycosides of these sugars should therefore be inactive if the structural sequence I is an absolute requirement for inhibition by acetamido sugars.

A progressive decrease in activity of *N*-acetyl-D-glucosaminides, which paralleled the change in size of substituents, was observed in the series



$$-O-C_2H_5 > -O-CH_3.$$

Subsitution of an *N*-butyryl group for *N*-acetyl of *N*-acetyl-D-glucosamine had little effect on activity whereas substitution of *N*-carboxymethyl or amino for *N*-acetyl markedly reduced or completely abolished activity. Replacement of $-CH_2OH$ at carbon atom 6 of *N*-acetyl-D-glucosamine by either —H or $-CH_2$-*O*-β-D-galactose also diminished the inhibitory activity. The 4-*O*-β-D-galactoside was inactive.

The assessment of significance to the weak reactivity displayed by L-fucose and 3-*O*-methyl-L-fucose is difficult at present. However, it is noteworthy that 2-*O*-methyl-L-fucose as well as all the D-fucose derivatives tested were inactive.

Certain sugars could not be tested for inhibitory activity because they ag-

Table 1. Inhibition of conglutination by carbohydrate. The test system contained 0.1 ml of conglutinin dilution, 0.1 ml of sheep erythrocyte-antibody-complement complex (*1*), and 0.3 ml of sugar in buffered saline. The mixture was incubated at 32°C for 30 minutes.

Sugar	Molarity required for inhibition
N-Acetyl-D-glucosamine	0.0004–0.0008
Chitobiose*	
N-Butyryl-D-glucosamine†	
6-*O*-β-D-Galactosyl-*N*-acetyl-D-glucosamine‡	0.0014–0.003
p-Nitrophenyl-2-acetamido-2-deoxy β-D-glucoside*	
Benzyl-2-acetamido-2-deoxy-α-D-glucoside§	
Benzyl-2-acetamido-2-deoxy-β-D-glucoside§	
Ethyl-2-acetamido-2,6-dideoxy-β-D-glusoside‡	0.060
Ethyl-2-acetamido-2-deoxy-β-D-glucoside‡	
3-Acetamido-3-deoxy-D-manno-D-galaheptose‡	
3-Acetamido-3-deoxy-D-glucose‡	
3-Acetamido-3-deoxy-D-galactose‡	
2-Acetamido-2-deoxy-D-xylose‡	
N-Carboxymethyl-D-glucosamine‡	
L-Fucose‖	
3-*O*-Methyl-L-fucose‖	
D-Glucosamine	Inactive
D-Glucosaminic acid¶	
Muramic acid#	
4-Acetamido-4-deoxy-D-glycero-L-galaoctose‡	
4-Acetamido-4-deoxy-D-glycero-L-idooctose‡	
3-Acetamido-3-deoxy-D-gluco-D-idoheptose‡	
3-Acetamido-3-deoxy-D-gluco-D-guloheptose‡	
Methyl-2-acetamido-2-deoxy-α-D-glucoside**	
Methyl-2-acetamido-2-deoxy-β-D-glucoside‡,**	
Methyl-3-acetamido-3-deoxy-D-manno-D-galaheptose‡	
2-Acetamido-2-deoxy-D-galactose‡	
2-Acetamido-2-deoxy-D-gulose‡	
2-Acetamido-2-deoxy-D-arabinose‡	
2-Acetamido-2-deoxy-D-ribose‡	
2-Acetamido-2-deoxy-D-mannose††	
2-*N*-Isopropyl-2-deoxy-L-glucosamine‡	
2-Acetamido-2-deoxy-D-sorbitol‡	
1-Acetamido-1-deoxy-D-glucitol¶	
2-*O*-Methyl-L-fucose‖	
D-Fucose‖	
Methyl-α-D-fucoside‖	
2-*O*-Methyl-D-fucose‖	
3-*O*-Methyl-D-fucose‖	
All common nonnitrogen containing mono- and disaccharides except L-fucose and 3-*O*-methyl-L-fucose	

Kindly supplied by: * O. Lüderitz, † K. Onodera, ‡ R. Kuhn, § H. K. Zimmerman, ‖ G. Springer, ¶ M. Wolfrom, # R. Gigg, ** F. Zilliken, †† S. Roseman.

glutinated the sensitized sheep erythrocytes treated with components of horse complement used to demonstrate conglutination (*1*). These sugars were lacto-*N*-tetraose, 2-acetamido-2-deoxy-D-altrose, *N*-acetyl-neuraminic acid, lacto-*N*-fuco-pentaose II, 2-acetamido-2-deoxy-L-fucose, and methyl-3-acetamido-3-deoxy-D-guloside. The mechanism of this agglutination is under investigation.

To determine whether the inhibitors were dissociating the erythrocyte-antibody-complement complex rather than the conglutination reaction, portions of the complex were extracted at 32°C for 15 minutes with either triethanolamine buffered saline solution (TBS) (*3*) or with this solution containing 0.02*M* L-fucose, 0.02*M* *N*-acetyl-D-glucosamine, or 0.02*M* galactose. The complexes were centrifuged, washed with the buffered saline solution, and tested for activity by incubation with various dilutions of purified conglutinin. No difference in activity was observed. Therefore extraction with either inhibitor (*N*-acetyl-D-glucosamine, L-fucose) or a non-inhibitor (D-galactose) had no significant effect on reactivity of the antigen-antibody-complement complex with conglutinin.

Conglutination of aggregated γ-globulin, protein-antiprotein immune aggregates, and polysaccharide-antipolysaccharide immune aggregates was inhibited by *N*-acetyl-D-glucosamine. Furthermore, conglutinated immune aggregates, washed with TBS, released conglutinin on extraction with the buffer solution containing 0.01*M* *N*-acetyl-D-glucosamine. These extracts had a higher ratio of conglutinin activity to total protein than extracts with ethylenediaminetetraacetic acid previously used to prepare purified conglutinin (*1*).

The data indicate that an acetamido sugar with structure I and possibly L-fucose take part in determining the specificity of the conglutination reaction. From the data we cannot discriminate between the possibility that the specific sugar moiety represents a portion of the conglutinin molecule, a portion of a complement component, or even a portion of the antibody molecule which has acquired the appropriate reactive structure after combination with complement.

MYRON A. LEON
RYUICHI YOKOHARI
Department of Surgical Research, St. Luke's Hospital, Cleveland, Ohio

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23 December 1964

Melatonin Synthesis in the Pineal Gland: Effect of Light Mediated by the Sympathetic Nervous System

Abstract. *Exposure to light reduces the ability of the rat pineal gland to synthesize melatonin and decreases the weight of the gland. When the sympathetic nerves to the pineal gland are cut, light no longer has an effect on melatonin synthesis or pineal weight. The response of the gland does not require that the gonads or the pituitary gland be present.*

Melatonin (5-methoxy *N*-acetyl tryptamine) (*1*) is synthesized in the mammalian pineal gland by the *N*-acetylation and subsequent *O*-methylation of serotonin (*2*). It has recently been shown that the activity of hydroxyindole-*O*-methyl transferase (HIOMT), the enzyme responsible for the *O*-methylation step, is influenced by illumination. Rats kept in constant darkness have 3 to 10 times as much melatonin-synthesizing-ability in their pineal glands as littermates kept in continuous light (*3*).

The effect of illumination on the pineal transferase, which is accompanied by smaller but consistent changes in pineal weight (*4*), is demonstrable within 24 hours (*5*) and appears to be specific, in that other enzymes in the pineal gland such as monoamine oxidase are unaltered (*3*). Since evidence has been presented that melatonin is secreted by the mammalian pineal gland (*6*) and inhibits ovary growth and the subsequent incidence of estrus in rats (*7*), it has been suggested that the light-induced inhibition of HIOMT activity may constitute a mechanism of neuroendocrine regulation of gonad function (*3*).

There are several ways in which information about lighting could reach the pineal gland of the rat. (i) Light could penetrate the skull and impinge directly upon the pineal. It has recently been shown that sufficient light penetrates the mammalian skull to activate photoelectric cells implanted within the brain (*8*). (ii) Light could act, through the hypothalamus and pituitary body or other neuroendocrine organs, to alter the level of a circulating hormone, which might in turn influence pineal HIOMT. Although the effect of light upon pineal weight does not require pituitary, gonad, adrenal, or thyroid function (*9*), ovarian hormones and the estrous cycle do influence the phospholipid content of the pineal gland (*10*). (iii) Information about lighting could be transmitted to the pineal via a neural route.

It has recently been demonstrated that the major, if not the only, innervation of the rat pineal gland is sympathetic, and consists of fibers whose cell bodies are in the superior cervical ganglia (*11*). It will be shown that the pathway by which information about lighting reaches the pineal gland involves the eyes and the sympathetic nervous system, and is independent of the pituitary body or gonads. The experiments described here will also demonstrate that it is possible to trace

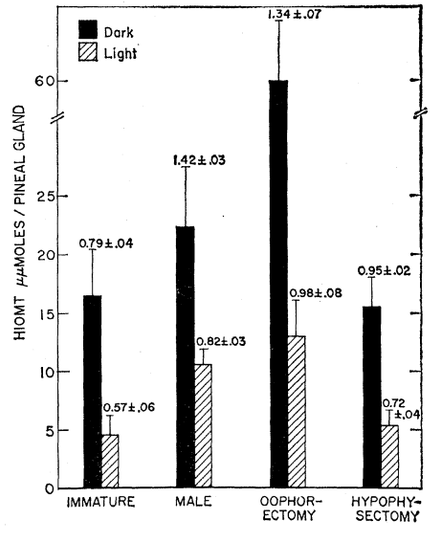

Fig. 1. The effect of constant light or darkness on the weight of the pineal gland and HIOMT activity in the rat. Immature 21-day-old female rats, and bilaterally-oophorectomized or hypophysectomized adult females were maintained under experimental lighting conditions for 6 days. Adult males were kept in light or darkness for 42 days. The HIOMT activity is expressed as micromicromoles of C^{14}-melatonin formed per whole pineal gland per hour. Average pineal weights ± standard error are given over the appropriate bars.