Meetings

Glucuronic Acid Research

The study of glucuronic acid in relation to many fields of biology and medicine has been a major continuing activity of Japanese investigators. This interest developed first from the work on the metabolism of drugs in which glucuronic acid conjugation is a frequently encountered feature, and then, with the ready availability of pure glucuronic acid (Chugai Pharmaceutical Company, Tokyo), from a widespread curiosity to examine its biological properties in almost every living situation. A measure of the magnitude of this research effort is the fact that for the last 4 years a research conference on this subject has taken place. I was privileged to be invited to attend the fifth Glucuronic Acid Research Conference which was held 2 and 3 June at Sankei Kaikan, Tokyo. At this conference and on a number of other visits in June to research laboratories in Japan, it was possible to exchange views and to acquire information regarding biochemical studies on glucuronic acid. My present purpose is to transmit this knowledge to American investigators who are working in this area of research.

The conference was opened by M. Ishidate, dean of the faculty of pharmaceutical sciences, University of Tokyo. It was attended by some 250 investigators invited from many research centers of Japan. Two novel features of the meeting were, first, the simultaneous use of two slide projectors and two screens to expedite illustration of the data, and second, the provision of a pocket radio transmitter which not only freed the speaker from the electrical wires which ordinarily chain him to the podium but gave pleasing sound amplification.

Forty-four original papers were presented. Thirteen were concerned with the biochemistry and physiology of glucuronic acid, four with its growth-promoting effect, twelve with detoxication of drugs, viruses, and toxins, and the remainder with the clinical use of glucuronic acid.

Biochemistry and Physiology

The conjugation of amines with glucuronic acid and the significance of this process was reported by Ishidate. Aromatic and aliphatic primary amines easily form N-glucuronides at room temperature in the presence of aqueous solutions of glucuronic acid or its alkaline salts. Crystalline N-glucuronides have been obtained of the following: aniline, toluidine, *p*-chloroaniline, monoacetyl-*p*-phenylenediamine, *p*-dimethylaminoaniline, *p*-phenylenediamine, sulfanilamide, sulfapyridine, benzylamine, β-phenylethylamine, ethylamine, isopropylamine, isobutylamine, and cyclohexylamine. A quantitative relation between amine metabolism and glucuronic acid excretion was observed when rabbits were given aniline, sulfanilamide, or sulfapyridine. Also, after the administration of *p*-dimethylaminoazobenzene to dogs, one of the urinary metabolites appeared to be the N-glucuronide of *p*-mono-methylaminoazobenzene. The studies have included the property of amino acids to form N-glucuronides. These can be detected by paper chromatography but, because of their lability, they have not yet been isolated. A reaction of glucuronic acid with diphtheria toxin takes place at *p*H 7.0 and 38°C with the gradual disappearance of toxicity. The formation of antiserum from the injection of this material was no different than in the case of formalin-inactivated toxin. It was therefore suggested that an amino group is the functional group of toxin and that this reaction depends upon formation of an N-glucuronide.

Ishidate then reviewed the two mechanisms which can explain glucuronic acid conjugation (UDPGA, β-glucuronidase) and found that information regarding the enzymatic mechanism of formation of N-glucuronide is incomplete.

Shimazono *et al.*, (department of biochemistry, University of Tokyo) reported that incorporation of phosphate into rat liver tissues was increased by the injection of D-glucuronate. From an analysis of fractions containing nucleotides and sugar phosphates, it was stated that this incorporation was most marked in the case of glucose-6-phosphate and *d*-glycerophosphate. In a carefully done enzymatic study of glucuronic acid, the following observations were made. D-Glucuronate was reduced to L-gulonate by TPN-gulonic dehydrogenase, and L-gulonate was oxidized following decarboxylation to L-xylulose by DPN-gulonic dehydrogenase. The formation of lactone from D-glucuronate or L-gulonate by soluble lactonase from cell supernatant was observed with the aid of hydroxylamine. The lactonase which acts on D-glucuronolactone was present in liver microsomes of various animals but not in human and monkey liver. L-Gulonolactone was formed easily from D-glucuronolactone by the action of TPN-gulonic dehydrogenase. A dehydrogenase present in microsomes produces ascorbate from L-gulonolactone.

Much discussion centered around in vivo effects of administered glucuronic acid in relation to carbohydrate metabolism, particularly in the liver. Thus,

Obara *et al.* drew attention to effects on the adrenal gland; Oda and Hara observed an increase in liver glycogen following glucuronolactone or xylulose injection; Imanaga stated that the increase in blood ammonia in patients with a portacaval shunt can be controlled with exogenous glucuronic acid; Oji studied urinary pentose in patients with liver disease and in animals with CCl_4 poisoning; Kobayashi *et al.* investigated the effect of glucuronic acid on experimental diabetes mellitus; Kurokawa and Yamagata reported experimental and clinical studies on a beneficial effect of glucuronic acid in diabetes; Masuda *et al.* investigated phosphorus metabolism in the liver of rabbits given carbon tetrachloride; and Kusuya *et al.* studied the effect of glucuronic acid on sugar assimilation.

With regard to β-glucuronidase, Tsukamoto *et al.* of Kyushu University proposed the use of *p*-nitrophenyl glucuronide as a substrate for both the hydrolytic and transfer activities of the enzyme. The enzyme was stated to catalyze the formation of *m*-aminophenyl glucuronide in a system in which benzoyl glucuronide was the donor substrate, and *m*-aminophenol, the acceptor molecule.

Growth-Promoting Effects

Several papers were presented which illustrate the variety of investigations into growth-promoting effects. Thus, Ogawa (National Institute of Genetics) observed that sodium glucuronate and glucuronic acid showed a significant growth-promoting effect on the early development of the embyro (*Triturus pyrrhogaster*) which was most prominent on the 13th day after fertilization (stage 32); Tamura (Tokyo Dental College) noted that the growth of chicks on a vitamin-B-deficient diet was promoted by glucuronolactone; and Wada *et al.* (Sapporo Medical College) reported that the prior administration of glucuronolactone to rats significantly promoted the growth of intraperitoneally transplanted Yoshida sarcoma cells, whereas the opposite effect occurred on the growth of subcutaneously transplanted Yoshida sarcoma.

Experimental and Clinical Studies on "Detoxication"

Although the papers on clinical and detoxication studies were the most numerous, yet, because of the great variety of conditions investigated they were not amenable to generalization.

The effect of exogenous glucuronic acid on the excretion of morphine glucuronide was thoroughly investigated by Hosoya and Otobe (Keio University School of Medicine). Thus, during the first 2 hours after administration of morphine, bound morphine appears earlier and in larger amounts in the urine of rabbits receiving glucuronic acid and morphine. Hosoya stated that these results seemed to indicate that exogenous glucuronic acid accelerates conjugation of morphine with glucuronic acid in the living body although it was by no means clear whether the exogenous glucuronic acid did conjugate with endogenous glucuronic acid by some unknown mechanism.

Shirai *et al.* (Kobe Medical College) extended his observations on the ethyl ester of glucuronic acid which augmented the formation of anthranilic acid glucuronide in the rabbit. He observed that the glucuronic acid ester is excreted in the urine more slowly than glucuronolactone.

Sawada (Kyushu University) stated that the direct-reacting bilirubin in cat bile was the glucuronide but that this substance was not produced by cat liver microsomes fortified with UDPGA.

Examples of the experiments and of the toxic compounds studied are Takahashi's (Kyoto) perfusion studies of conjugation by livers damaged with carbon tetrachloride; Tsumoo's (Showa Medical College) study of the effect of 1-phenyl-2-methylaminopropane and ephedrine on blood pressure and respiration in the urethanized rabbit; Tanuora's

(Keio University) work on the isolated surviving frog heart, hypnotic and lethal actions of barbiturate, and changes of oxygen consumption of rat brain tissue homogenate; Harashima's (Keio University) observations on the urinary excretion of glucuronic acid in rabbits experimentally exposed to benzene and carbon disulfide; Ito's study on the detoxication of 2,4-dinitrophenol, and Takatsu's (University of Tokyo) work on the effect of sodium glucuronate on the LD_{50} of *Shigella* endotoxin, phenol, pyramine, and noradrenaline in the young mouse.

In the field of virology, Ogasawara *et al.* (Nagoya University) studied the effects of glucuronic acid salts on the activity of influenza virus PR8 and of Newcastle disease virus in producing pyrogenic skin lesions and pulmonary consolidation. On the whole, infectivity, hemagglutinin, and antigenicity of these two viruses were not affected significantly, but the toxicity (skin lesion, pulmonary consolidation) was prevented by preliminary treatment with glucuronolactone. Coto *et al.* (University of Tokyo) observed the in vitro inhibition by glucuronic acid of mouse hepatitis virus, rabies virus, and Japanese encephalitis virus, with regard to their ability to infect the host mice.

Clinical studies included work on the effects of glucuronic acid on steroid hormone excretion during pregnancy (Moriyama *et al.*); studies on glucuronic acid metabolism of newborn infants (Iwanami *et al.*); three separate investigations on glucuronic acid interrelationships with adrenal cortical function (Tokita *et al.*, Oshima *et al.*, and Kawai *et al.*); two reports on a therapeutic effect of glucuronolactone in diabetes mellitus (Katsuki *et al.* and Matsuoka *et al.*); the treatment of epidemic hepatitis with glucuronic acid (Kosaka *et al.*); and the influence of glucuronolactone on experimental liver injury induced by *Penicillium islandicum* Sopp poisons (Suzuki *et al.* and Uraguchi *et al.*).

It appears that with glucuronolactone occupying a central position in the glucuronic acid pathway of glucose metabolism, the basis of interpretation of the results of experimental and clinical work with exogenous glucuronolactone is to be found mainly in the field of carbohydrate metabolism. Nevertheless, "detoxifying" effects of glucuronolactone or glucuronic acid may be explained on a direct basis, such as in N-glucuronide formation, or may result indirectly from alterations in the dynamics of the glucuronic acid pathway. The evidence indicates that glucuronolactone is indeed a physiological nutrient or metabolite and its study provides many indications of rewarding further critical investigation.

The address by W. H. Fishman (Tufts University School of Medicine) was published in monograph form (*Biochemistry of Glucuronic Acid Lectures in Japan*, published by the Glucuronic Acid Research Conference, University of Tokyo, 1959). Worthy of mention was the report of the discovery of a new 3-ketoheptose phosphate by Sie, Nigam, and Fishman, and the presentation of a concept of the role of β-glucuronidase in which the enzyme is believed to be associated with processes of cell and tissue differentiation rather than with cell division or growth per se.

I recall with great pleasure both the many enjoyable events of the conference and the lectures which it was my privilege to deliver at a number of Japanese institutions of higher learning. The kind and sincere hospitality of my hosts are herewith acknowledged with many thanks. My appreciation is expressed also to the National Science Foundation for granting a travel award.

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Forthcoming Events

January

6–8. Northeastern Weed Control Conf., 14th annual, New York, N.Y. (M. G. Wiltse, Chairman, Public Relations Committee, Dow Chemical Co., 916 Shoreham Bldg., 15 and H Sts., NW, Washington 5, D.C.)

7–10. Radioactive Isotopes, 4th intern. symp., Bad Gastein, Austria. (R. Hofer, Isotopen-Laboratorium, II. Medizinische Universitäts Klinik, 13, Garnisongasse, Vienna 9, Austria.)

8–11. Sanitary Engineering Conf., ASCE, Cincinnati, Ohio. (E. S. Kirkpatrick, ASCE, 33 W. 39 St., New York 18.)

11–13. American Acad. of Allergy, Hollywood-by-the-Sea, Fla. (J. O. Kelley, 756 N. Milwaukee St., Milwaukee 2, Wisc.)

11–13. Arctic Geology, 1st intern. symp. Calgary, Alberta, Canada. (D. W. R. Wilson, Arctic Symposium Committee, P.O. Box 100, Calgary, Alberta, Canada.)

11–13. Reliability and Quality Control, natl. symp., Washington, D.C. (N. S. Hibshman, AIEE, 33 W. 39 St., New York 18.)

11–15. Society of Automotive Engineers, annual, Detroit, Mich. (R. W. Crory, Meetings Operation Dept., SAE, 485 Lexington Ave., New York 17.)

11–25. Effects of Atomic Radiation, New York, N.Y. (R. Appleyard, Scientific Committee on the Effects of Radiation, United Nations, New York 17.)

12–15. Society of Plastics Engineers, 16th annual conf., Chicago, Ill. (T. A. Bissell, SPE, 65 Prospect St., Stamford, Conn.)

14–18. American Inst. of Mining, Metallurgical, and Petroleum Engineers, annual, New York, N.Y. (E. O. Kirkendall, AIMMPE, 29 W. 39 St., New York 18.)

17–30. Bahamas Medical Serendipity Conf., 2nd, Nassau. (B. L. Frank, P.O. Box 4037, Fort Lauderdale, Fla.)

18–21. American Astronautical Soc., 6th annual, New York, N.Y. (A. P. Mayernik, AAS, 6708 53 Rd., Maspeth 78, N.Y.)

19–21. American Meteorological Soc., 40th annual, Boston, Mass. (K. C. Spengler, 3 Joy St., Boston.)

19–21. Congenital Malformations, CIBA symp. (by invitation only), London, England. (G. E. W. Wolstenholme, 41 Portland Pl., London, W.1.)

21–23. American College of Surgeons, Louisville, Ky. (H. P. Saunders, 40 E. Erie St., Chicago 11, Ill.)

23–28. American Acad. of Orthopedic Surgeons, Chicago, Ill. (J. K. Hart, 116 S. Michigan, Chicago 3.)

24–29. American Rocket Soc., Princeton, N.J. (J. J. Harford, ARS, 500 Fifth Ave., New York 36.)

25–28. Institute of the Aeronautical Sciences, 28th annual, New York, N.Y. (IAS, 2 E. 64 St., New York 21.)

25–28. Plant Maintenance and Engineering Show, Philadelphia, Pa. (R. S. Wolcott, Clapp & Poliak, 341 Madison Ave., New York 17.)

25-29. Stress Measurement Methods, symp., Tempe, Ariz. (P. K. Stein, Strain Gage Readings, 5602 East Monte Rosa, Phoenix, Ariz.)

(See issue of 13 November for comprehensive list)

New Products

The information reported here is obtained from manufacturers and from other sources considered to be reliable. Neither Science *nor the writer assumes responsibility for the accuracy of the information. All inquiries concerning items listed should be addressed to the manufacturer. Include the department number in your inquiry.*

■ MAGNETIC-TAPE CONVERTER makes possible direct use of digital magnetic tape as input to automatic graphical plotters. Solid-state switching circuitry is used with patchboard programing. Provision is included for omitting records that contain specified characters. (Benson-Lehner Corp., Dept. Sci254, 11930 West Olympic Blvd., Los Angeles 64, Calif.)

■ RATIO STANDARD combines a d-c divider and a ratio transformer. Model 1001 handles a-c input voltages of 0.35 times the frequency from 50 to 10,000 cy/sec. Model 1002 handles inputs of 2.5 times frequency from 30 to 1000 cy/sec. Input resistance of the d-c section is 10,000 ohms, and power rating is 5 watts. Linearity is said to be 0.0001 percent for a-c and 0.001 percent for d-c. (Gertsch Products, Inc., Dept. Sci255, 3211 South La Cienezo Blvd., Los Angeles 16, Calif.)

■ FREQUENCY CALIBRATOR furnishes standard frequencies from 10 kcy/sec to 1000 Mcy/sec with short time stability of $1/10^7$. The instrument also provides timing markers at decade intervals from 0.1 to 100 μsec. An internal crystal oscillator is coupled to a 2-to-1 multiplier followed by a 10 Mcy/sec buffer that drives a series of multivibrators with fundamentals of 1 Mcy/sec and 100 and 10 kcy/sec. (General Radio Co., Dept. Sci259, West Concord, Mass.)

■ RESISTORS of radial-lead and axial-lead encapsulated types are said to be available with absolute accuracies from ± 0.005 to ± 0.1 percent and stability ± 0.003 percent per year. Accuracy over the temperature range $-30°$ to $+85°$C is said to be ± 0.02 percent, and matched sets which track within ± 0.005 percent from $-45°$ to $+85°$ can be provided. Resistances from 1 ohm to 4 megahom are available. (Julie Research Laboratories, Dept. Sci260, 556 W. 168 St., New York 32, N.Y.)

■ AIR MONITORING SYSTEM is said to eliminate interference of natural radon with measurement of long-half-life emitters by measuring the ratio of beta-gamma to alpha activity. Change in this ratio, essentially constant in natural radron-thoron progeny, indicates presence of other than normal background. Ratio rise indicates long-half-life predominantly beta activity; ratio fall, pre-