"Picard" and schlicht functions and other related topics.

It is written for the research worker in complex variable theory. Emphasis is placed on "best possible" results, on the comparison between alternative methods of proof, on establishing "existence theorems" with a maximum of generality and on precise inequalities. Informal comments such as "this theorem is difficult (and the reader may ignore it if he wishes)" enliven the reading. Though the non-specialist will find the standard treatises of Titchmarsh and Copson sufficiently detailed and perhaps better balanced, the specialist will want Professor Littlewood's book for the intimate and critical perspective which it gives into the structure of complex variable theory.

GARRETT BIRKHOFF

SPECIAL ARTICLES

GLUCURONIC ACID AS A MEASURE OF THE ABSORPTION OF PENICILLIN

THE coupling of such compounds as resist the oxidative processes of the body, with normal metabolic compounds, constitute a primary defense mechanism. The animal organism utilizes a variety of substances for conjugation, principally glycine, glucoronic acid, sulfuric acid, cysteine, glutamine, acetic acid, ornithine and the methyl group. The main source of glucuronic acid is the carbohydrate store in the body, but it can also be derived from glucogenetic amino acids.[1, 2]

That organic compounds can be conjugated in the mammalian body is a fact first recognized by Baumann.[3] Since his early recognition of this fact, it has since been demonstrated by Deichmann *et al.*[4] that many other compounds undergo conjugation in this manner.

Enklewitz[5] demonstrated that the reducing substance found in the urine after the ingestion of amidopyrine is a conjugated glucuronic acid complex. Tsunoo[6] did extensive work with ethynal and found that the body hydrolyzes it to furylacrylic acid, which it conjugated with glycine, and p-hydroxy-phenylurea, which it combines with glucuronic acid. Horn[7] reported that dimethylanaline in the rabbit is converted to p(mono)-methylamino-phenol and is excreted in combination with glucuronic acid. In dogs, Horn found that the same compound is changed to o-aminophenol.

In view of these reported findings this investigation was undertaken to determine whether a similar conjugation takes place between penicillin and glucuronic acid and if so whether the glucuronic acid determination in urine may be used as a measure of the absorption of penicillin. It was further hoped this study would offer additional information in determining the mode of action of penicillin.

EXPERIMENTAL

Male New Zealand rabbits were employed weighing approximately 2,400 to 3,200 grams. They were maintained on a diet consisting chiefly of a standard dry commercial feed (Purina rabbit pellets) and small daily portions of fresh carrots and cabbage. The treated animals received intravenous doses of penicillin sodium varying from 50,000 to 200,000 Oxford units (300 μ/mg penicillin salt) and were placed in metabolism cages for the collection of urine. The cages were designed to avoid the contamination of feces with the excreted urine. Urine was collected for control over 24-hour periods for one week prior to treatment. Glucuronic acid and organic sulfate determinations were made daily.

Fig. 1 demonstrates the influence of penicillin on

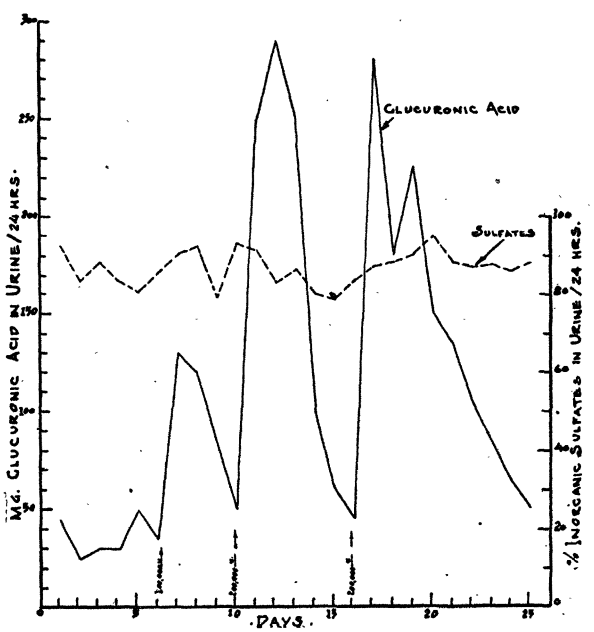

FIG. 1. Effect of penicillin on the excretion of glucuronic acid and inorganic sulfates in the urine.

[1] Anthony Ambrose and Carl P. Sherwin, "Detoxication Mechanisms," Vol. 2, p. 377, 1933.

[2] Benjamin Harrow and Carl P. Sherwin, "Detoxication Mechanisms," Vol. 4, p. 263, 1935.

[3] E. Baumann, "Weber Gepaarte Schwefelsauren im Oganismus," *Arch. f. d. ges. Physiologie*, 13: 285, 1876.

[4] Wm. Deichmann and G. Thomas, *Jour. of Ind. Hygiene and Toxicology*, 25: 286, 1943.

[5] M. Enklewitz and M. Lasker, *Jour. Biol. Chem.*, 110: 443–56, 1935.

[6] S. Tsunoo, *Jour. Biochem.* (Japan), 21: 409–16, 1935.

[7] F. Horn, *Z. f. Physiol. Chem.*, 242: 23–28, 1936.

the glucuronic acid content in urine in a typical animal experiment. The time of penicillin treatment is indicated. A twenty-five-day test period was used.

The glucuronic acid of the untreated animals was found to be within the normal range for rabbits, as set forth by Deichmann.[8] However, 24 hours after penicillin treatment, a marked increase in glucuronic acid was quantitatively demonstrable by the methods of both Deichmann[8] and Hanson.[9]

Analyses for solids, specific gravity, pH, sugar and albumin were also daily made, but since there was no significance in the data they are not recorded.

For the purpose of comparison, the excretion of penicillin as organic sulfates was also determined and the results recorded as per cent. of inorganic to total sulfates. The method of Treon and Crutchfield[10] was used for these determinations. Since there was no definite trend in the values obtained other than normal variation, it is concluded that penicillin does not influence the *in vivo* excretion of sulfates.

RESULTS

The normal 24-hour excretion of glucuronic acid in rabbit urine maintained on a diet of Purina rabbit chow, fresh carrots and cabbage ranges from 25 to 60-mg.

Following treatment of rabbits with penicillin sodium, a marked increase in glucuronic acid was noted. The treated animals gradually returned to normal.

Tests were made to determine whether or not penicillin sodium itself gives a color reaction characteristic of the naptha-resorcinol-glucuronic acid method of both Deichmann[8] and Hanson.[9] The results of the investigation in both instances were negative.

DISCUSSION

It is the general opinion of most investigators that approximately 60 per cent.[11] of penicillin administered can be recovered from the excreted animal urine. Since in this investigation it was found that there was an immediate sharp increase in the glucuronic acid content of rabbit urine following the intravenous administration of penicillin, it would appear likely that some part of the unaccounted-for 40 per cent. of penicillin normally excreted from the animal body conjugated with glucuronic acid.[4]

The data suggest the possibility of using the quantitative determination of glucuronic acid in urine to detect the presence and the extent of absorption of penicillin.

It is further possible that, since commercial penicillin contains certain impurities, the increased glucuronic acid in urine may be due to the impurities of penicillin rather than penicillin itself. The experiments will be extended with more highly purified penicillin.

D. PERLSTEIN
H. E. WRIGHT
A. J. LIEBMANN
I. DORRELL,
Technical Assistant
SCHENLEY RESEARCH INSTITUTE INC.,
LAWRENCEBURG, IND.

NON-ACID-FAST FORMS OF THE MYCOBACTERIUM OF HUMAN LEPROSY*

IN 1939, the writer had the opportunity of staining several smears of material obtained at the Branch Laboratory of the New York State Department of Health from the nasal septum of a Mexican who had been diagnosed as an early case of leprosy.[1] The smears were stained by the author's Triple Stain method[2] which reveals non-acid-fast forms of *Mycobacterium tuberculosis* not disclosed by the usual Ziehl-Neelsen technic. When this method is applied to material from tuberculous lesions or cultures, acid-fast tubercle bacilli stain red, non-acid-fast forms (rods, granules, and the recently demonstrated zoogleal forms)[3] stain blue, while other organisms, tissue cells, etc., form a light green background.

The two leprosy smears stained in this manner showed acid-fast rods, and a few non-acid-fast forms. As soon as the patient became aware of the diagnosis of his affliction, he made an escape from supervision, and no further material could be obtained.

Recently, however, Dr. Frank Combes, professor of dermatology at New York University and chief of the Dermatology Service at Bellevue Hospital, suggested that material from cases of leprosy might reveal interesting non-acid-fast forms if stained by the author's Triple Stain technic. He kindly arranged to have a number of unstained fixed smears of nasal

* This work was supported by a grant from the Lessing J. Rosenwald Foundation.

[1] Dr. Morton C. Kahn informs me that in 1929 he visited Mahaika Leprosarium in British Guiana after completing his studies on the development of the tubercle bacillus, as he felt that non-acid-fast forms might be concerned in the pathology of leprosy. The finding of so many non-acid-fast saprophytes in the nasal cavity caused the work to be discontinued, and there was insufficient time for skin biopsies.

[2] E. Alexander-Jackson, SCIENCE, 99: 307, 1944.

[3] E. Alexander-Jackson, *Annals of the New York Academy of Sciences* (in press).

[8] Wm. Deichmann, *Jour. Lab. and Clin. Med.*, 28: 770, 1943.

[9] S. W. Hanson, G. T. Funch Mills and R. T. Williams, *Biochem. Jour. England*, 38: 3, 274, 1944.

[10] Treon and Wm. Crutchfield, Jr., *Ind. Eng. Chem., Anal. Ed.*, 14: 119, 1942.

[11] C. K. Rammelkamp and C. S. Keefer, *Jour. Clin. Invest.*, 22: 425, 1943.