XIX. THE BIOCHEMISTRY AND PHYSIOLOGY OF GLUCURONIC ACID.

IV. (a) THE OCCURRENCE OF CONJUGATED GLUCURONIC ACIDS IN THE ANIMAL BODY.

(b) OBSERVATIONS ON THE CONJUGATION OF *d*- AND *l*-BORNEOL.

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(Received November 9th, 1933.)

GLUCURONIC ACID IN NORMAL ANIMAL URINE.

FOLLOWING the recognition of the part played by glucuronic acid in detoxication mechanisms, its possible occurrence in normal urine was suggested by Hoppe-Seyler [1882] and was further considered by Flückiger [1885] in the course of his investigations of the copper-reducing substances of human and animal urines. Several early observers [Haas, 1876 and others] had shown that normal human urine is slightly laevorotatory and it was probable that this laevorotation was due to the presence of conjugated glucuronic acids, which are all laevorotatory. Proof of the occurrence of glucuronic acid in normal urine was provided by Mayer and Neuberg [1900], who isolated it from human urine as the *p*-bromophenylhydrazine derivative. These workers concluded that glucuronic acid occurs in normal human urine conjugated mainly with phenol and cresol and to a lesser extent with indoxyl and skatoxyl. It had previously been suggested by Hoppe-Seyler [1882] that glucuronic acid might be excreted in normal urine as the conjugated indoxyl compound, and Mester [1888] found a laevorotatory and copper-reducing substance in the urine of dogs after administration of skatole, which he regarded as the hypothetical skatoxylglucuronic acid. Neuberg and Niemann [1905] also suggested that urea-glucuronic acid, which they had synthesised, might occur in normal urine, and Pollecoff [1924] stated that glucuronic acid is a probable constituent of urochrome, the yellow pigment of urine, isolated from human urine and carefully purified. Tollens [1910] has emphasised the fact that man excretes twice as much conjugated glucuronic acid daily as ethereal sulphate and even within the limits of normal behaviour much more glucuronic acid can be excreted daily than of ethereal sulphate. The quantity of glucuronic acid in normal urine has been determined by several workers and values from 4 to 25 mg. per 100 ml. have been quoted (see Table I).

With reference to the occurrence of glucuronic acid in the urine of other animals little can be found in the literature. Hagemann [1882] states that it is probably a part of the reducing substance he obtained from horse urine, while Kawanishi [1932] has estimated it in rabbit urine (see Table I). We have detected

Table I.

Observers	mg. glucuronic acid per 100 ml. urine	
	Normal human urine	Rabbit urine
Boku and Kin [1931]	13	—
Kawanishi [1932]	5 to 10	4 to 15
Mayer and Neuberg [1900]	4	—
Sauer [1930]	15 to 19	—
Tollens and Stern [1910]	25	—

glucuronic acid in the normal urine of all animals tested, using the Tollens naphthoresorcinol test, and we have confirmed its presence in human urine by the same test (see Table II).

Table II.

Animal	No. tested	Naphthoresorcinol test
Man	15	+
Dog	30	+
Cat	18	++ +
Mouse	6	++
Guinea-pig	2	+

It is interesting to note that cat urines always give a much stronger positive test than the urine of other animals listed, and this strong test is associated with an intense Obermayer indoxyl reaction. It would seem probable that cat urine normally contains a relatively greater amount of the hitherto undescribed indoxylglucuronic acid than the urine of other animals tested, and it is therefore a likely source for isolating this compound. The conjugated glucuronic acids of normal urine (*i.e.* phenyl, cresyl, indoxyl and skatoxyl) have not been isolated from such urine, but phenylglucuronic acid has been isolated from the urine of sheep after administration of phenol [Salkowski and Neuberg, 1906]; cresylglucuronic acid has been isolated as a double barium salt with phenylsulphuric acid from the urine of dogs fed with *p*-cresol [Neuberg and Kretschmer, 1911]; and Neuberg and Schwenk [1917] have isolated a double barium salt of indoxylglucuronic and -sulphuric acids from urine after the feeding of indole. It is of interest to point out that the non-fermentable sugar of urine, which was shown by West *et al.* [1932] to increase during intestinal stasis in the dog, is probably in part glucuronic acid, which itself is not fermented by yeast.

GLUCURONIC ACID IN NORMAL ANIMAL BLOOD.

Scanty reference has been made in the literature to the occurrence of glucuronic acid derivatives in blood, but their presence there follows from their elimination by the kidney, since it is shown that the kidney does not synthesise conjugated glucuronic acids [Hemingway *et al.*, 1934]. Glucuronic acid, as the *p*-bromophenylhydrazine derivative, has been isolated from bovine blood by Mayer [1901], who states that it is probably constantly circulating in human blood. Letsche [1907] states that amongst the reducing substances in blood of carbohydrate nature are glucose and glucuronic acid; the presence of the latter was deduced from orcinol- and phloroglucinol-HCl tests and from the isolation of a hydrazone. Stepp [1919] showed it to be present in normal human blood by means of the naphthoresorcinol test, and Ørskov [1932], in a study of the ether-soluble acids of blood, claims that minute quantities of conjugated glucuronic acids are present. Lépine and Boulud [1905; 1906] have made estimations of the conjugated glucuronic acid in arterial and venous blood, in serum

and in the corpuscles, and find as much as 14 to 47 % of the blood-sugar to be conjugated glucuronic acids; they base their values on reducing powers and rotations, but, owing to interfering substances (now known to be present in blood) which affect these methods, these values cannot be accepted without further investigation.

We have confirmed the fact that human blood contains glucuronic acid, using the naphthoresorcinol reaction and find that dog and cat bloods also give a slight positive test. The test was carried out on blood after deproteinising with sodium tungstate and sulphuric acid. The test was then compared with a blank using the deproteinising agents and water and with a known positive test on blood containing bornylglucuronic acid. We have also found that the blood

Table III.

Animal	Method	Observer
Man	Naphthoresorcinol test	Stepp [1919] and this investigation
Dog	"	This investigation
Cat	"	"
Ox	Isolation of <i>p</i> -bromophenylhydrazide	Mayer [1901]

and serum of man and the dog give very intense naphthoresorcinol tests for glucuronic acid, 10 minutes after oral administration of 2 g. of borneol, from which it seems that the detoxication of borneol begins to take place rapidly.

THE STEREOCHEMISTRY OF BORNYLGLUCURONIDE IN RELATION TO ITS FORMATION IN THE BODY.

All natural glycosides possess the β -type of linkage, and this may now be regarded as true also for glucuronides synthesised *in vivo*. Bornylglucuronide, from the urine of dogs fed with borneol, has been shown by Hämäläinen [1911] to be a β -glucosidic compound, since it is hydrolysed by the β -glucosidase of emulsin. However, Miwa [1932] stated that not all β -glucuronides are hydrolysed by this β -glucosidase. Positive results have been obtained by Neuberg and Niemann [1905] with phenylglucuronide and euxanthic acid, by Hämäläinen [1911] with *l*-camphorylglucuronide, by Hildebrandt [1905] with syringa- and vanillin-glucuronide, by da Cruz [1930] with menthylglucuronide, and by Ishidate [1929] with *d*-camphorylglucuronide and menthylglucuronide. Negative results were obtained by Schmiedeberg and Meyer [1879] with *d*-camphorylglucuronide, by Sera [1914] with orcinol- and vanillin-glucuronides, and by Miwa [1932] with menthylglucuronide. The varying behaviour of emulsin towards conjugated glucuronic acids is explained by Miwa [1932] to be partly due to the activity of the enzyme which seems essentially dependent on method of preparation and degree of ageing. In nature all derivatives of glucose are without exception derivatives of *d*-glucose, and the naturally formed glucuronides also fall into this category. Bornylglucuronide on hydrolysis gives *d*-glucurone and *d*-glucuronic acid. These facts therefore show that the glucuronide present in urine after ingestion of borneol is β -bornyl-*d*-glucuronide. However, there still remains to be considered the optical configuration of the bornyl residue and its influence, if any, on conjugation.

It was found by Magnus-Levy [1907] that when pure *d*- or *l*-borneol was fed separately to dogs or rabbits, pure *d*- or *l*-bornylglucuronide was excreted in the urine and conjugation took place equally well with either isomeride. The conjugated glucuronic acids were isolated as the sodium salts (sodium β -*d*-bornyl-*d*-glucuronide, $[\alpha]_D - 36.7^\circ$ in water, sodium β -*l*-bornyl-*d*-glucuronide, $[\alpha]_D - 66.7^\circ$

in water). We have fed pure *l*-borneol¹ to dogs and have recovered from the urine pure β -*l*-bornyl-*d*-glucuronide, $[\alpha]_{5461} - 74.6^\circ$ in water. On the other hand, as is shown in Table IV, we find that when a mixture of *d*- and *l*-borneol is fed to dogs preferential conjugation of the *d*-isomeride occurs, and da Cruz [1930], on feeding inactive menthol to dogs found the menthol after liberation from the conjugated derivative to be optically active. In the course of many of our feeding experiments the rotations of the borneol fed, the glucuronide excreted and the borneol recovered from it after hydrolysis were noted (Table IV).

Table IV.

	$[\alpha]_{5461}$	% <i>d</i> -isomeride
Borneol fed	+20° (EtOH)	73.7
Bornylglucuronide excreted	-47.9 (water)	86.7 } Mean increase
Borneol recovered after hydrolysis	+30 (EtOH)	85.5 } 12.4 %
Borneol fed	+26.5 (EtOH)	81.4
Bornylglucuronide excreted	-45.6 (water)	93.0 } Mean increase
Borneol recovered after hydrolysis	+33 (EtOH)	89.3 } 9.8 %
Calculated from figures quoted by Quick [1927]		
	$[\alpha]_D$	% <i>d</i> -isomeride
Borneol fed	+5°	56.7
Bornylglucuronide excreted	-48	66; increase 9.3 %
Borneol recovered after hydrolysis	No observation	No observation

SUMMARY.

1. A brief summary including new observations is given of the normal occurrence of glucuronic acid in the urine and blood of various animals.
2. A stereochemical study of the conjugation of *d*- and *l*-borneol with glucuronic acid *in vivo* reveals a preferential conjugation of the *d*-isomeride.

The expenses of this work were in part defrayed by a grant from the Medical Research Council. One of us (R. T. W.) is indebted to the Council for a whole time assistance grant.

¹ *l*-Borneol fed had $[\alpha]_{5461} - 42.7^\circ$ in alcohol; Mitchell [1925] gives the rotations of *d*- and *l*-borneol as $[\alpha]_{5461} \pm 42.2^\circ$ in alcohol.

REFERENCES.

- Boku and Kin (1931). *J. Chosen Med. Assoc.* **21**, 67.
da Cruz (1930). *Comp. Rend. Soc. Biol.* **105**, 815.
Flückiger (1885). *Z. physiol. Chem.* **9**, 323.
Haas (1876). *Centralbl. med. Wiss.* **149**.
Hagemann (1882). *Pflüger's Arch.* **28**, 506.
Hämäläinen (1911). *Skand. Arch. Physiol.* **23**, 297.
Hemingway, Pryde and Williams (1934). *Biochem. J.* **28**, 136.
Hildebrandt (1905). *Hofmeister's Beitr.* **7**, 438.
Hoppe-Seyler (1882). *Z. physiol. Chem.* **7**, 425.
Ishidate (1929). *J. Pharm. Soc. Japan*, **49**, 336.
Kawanishi (1932). *Mitt. med. Akad. Kyoto*, **6**, 289.
Lépine and Boulud (1905). *J. Physiol. Path. Gen.* **6**, 775.
— (1906). *J. Physiol. Path. Gen.* **8**, 581.
Letsche (1907). *Z. physiol. Chem.* **53**, 110.

- Magnus-Levy (1907). *Biochem. Z.* **2**, 319.
- Mayer (1901). *Z. physiol. Chem.* **32**, 518.
- and Neuberg (1900). *Z. physiol. Chem.* **29**, 256.
- Mester (1888). *Z. physiol. Chem.* **12**, 142.
- Miwa (1932). *Acta Phytchim.* **6**, 155.
- Mitchell (1925). *J. Chem. Soc.* **127**, 208.
- Neuberg and Kretschmer (1911). *Biochem. Z.* **36**, 15.
- and Niemann (1905). *Z. physiol. Chem.* **44**, 97.
- and Schwenk (1917). *Biochem. Z.* **79**, 387.
- Ørskov (1932). *Skand. Arch. Physiol.* **63**, 255.
- Pollecoff (1924). *Biochem. J.* **18**, 1252.
- Quick (1927). *J. Biol. Chem.* **74**, 331.
- Salkowski and Neuberg (1906). *Biochem. Z.* **2**, 308.
- Sauer (1930). *Klin. Woch.* **9**, 2350.
- Schmiedeberg and Meyer (1879). *Z. physiol. Chem.* **3**, 422.
- Sera (1914). *Z. physiol. Chem.* **90**, 258.
- Stepp (1919). *Z. physiol. Chem.* **3**, 422.
- Tollens (1910). *Z. physiol. Chem.* **67**, 138.
- and Stern (1910). *Z. physiol. Chem.* **64**, 39.
- West, Lange and Peterson (1932). *Biochem. J.* **26**, 1728.