GLYCOCOLL AND TOTAL MONO-AMINO-ACIDS IN PATHOLOGICAL URINES

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The present investigation was undertaken with the object of ascertaining whether there were any abnormal changes in the urinary amino-nitrogen of gouty patients. Since its commencement in 1904, papers upon the same subject have been published by Ignatowski¹, and Embden and Reese², and Plaut and Reese³, and Abderhalden and Barker,⁴ and F. Erben⁵, and Neuberg and Manasse⁶. Ignatowski¹ found amino-acids to be present in appreciable quantities in the urines of patients suffering from gout, pneumonia, and leuchaemia, while in normal urines only minute traces were observed. Embden and Reese, on the other hand, record the presence of considerable quantities of amino-acids in normal urine. Plaut and Reese fed animals and men on *r*-alanin, and Schittenhelm and Katzenstein¹¹ on *i*-alanin, and recovered from the urine a large percentage of the amount administered, in the form of *l*-alanin-naphthalin-sulpho-acid. Lipstein⁷ has since demonstrated similar amounts of amino-acids in gouty urines to those obtained by Embden and Reese from normal urines.

My present communication concerns itself with the appearance of glycocoll in the urine of children as well as of healthy adults, and with the amino-acid excretion in individuals under varying conditions, rather than with a number of isolated cases. It tends, to some extent, to confirm the findings of Embden and Reese with regard to normal urine. It records the variations in the amount of glycocoll excreted by gouty individuals, but does not satisfactorily clear up the question as to whether the amino-acids are excreted in the free state—thus escaping in small quantities through the renal filter—or whether they appear in the urine in combination with other substances, their ‘pairing’ properties being used for the elimination of bodies otherwise difficult

to excrete. Such questions, as well as those relative to the interesting synthesis and decomposition of amino-acids during the process of metabolism are left for discussion in later papers.

METHOD

The introduction by Fischer and Bergell of the reaction of amino-acids with β -naphthalin sulphochloride opened the way for a much more exact method of estimating urinary amino-acids than was possible under the earlier era.

Abderhalden and Bergell⁴ elaborated a quantitative method for the application of the reaction to urine, and Ignatowski modified the process so that it could be used in hospital laboratories. In their papers, the exact details of procedure and the properties of the several amino-naphthalin compounds are fully detailed, so that it is here needless to repeat them. Embden and Reese further altered the method, in that they more effectually removed the hippuric acid with 20 per cent. acetic ether, and then increased the alkalinity of the fluid in which the final precipitation of the amino-compounds takes place. This latter precaution, they claim, accounts for the increase of the final amino-products in their estimations.

There are one or two points upon which I must remark in this connection. First the urine must be treated in the fresh condition. The following extreme example, culled from a number of determinations, illustrates this necessity.

3,000 c.c. of normal urine were collected, and divided into two portions of 1,500 c.c. :

1,500 c.c. treated immediately	yielded 0,1840 gm. amino-naphthalin acids.
1,500 c.c. " three months later	" 0,0536 " " "

A second sample of 3,000 c.c. of abnormal urine was obtained, and divided into similar portions :

1,500 c.c. treated immediately	yielded 0,6073 gms. amino-naphthalin acids.
1,500 c.c. " three months later	" 0,0510 " " "

The smaller figures appear to correspond to the possible errors of the method, as ascertained by Abderhalden and Schittenhelm⁶ with regard to Frey's work upon glycocoll in cartilage.

Next, the alkalinity during the final precipitation is at present difficult to estimate. In my earlier estimations, I unintentionally used too much alkali, and obtained higher results than those of Ignatowski. Embden later made the solution still more alkaline, and found still larger amounts of animo-compounds. Accuracy is certainly endangered by excessive alkalinity, which permits the formation of naphthalin amides, which have to be removed at a later stage. Abderhalden and Schittenhelm distinctly consider that the use of too much alkali leads to the formation of large quantities of naphthalin-sulpho-amides, which are not too easily removed. Embden and Reese, on the other hand, deem it of little consequence, since the amides can be separated from the naphthalin-sulpho-amides at a later stage. My own experience leads me to regard the excessive alkalinity of the solution as a doubtful procedure, since the separation of the amides introduces complications which are not easy to obviate with accuracy, and introduces a factor—not hitherto allowed for—which may occasion the splitting off of glycocoll, etc., from substances with which it is intimately combined. At best, the present methods, and therefore the results, cannot be considered as more than roughly quantitative.

The method I have adopted is that used by Ignatowski and others, together with these precautions.¹⁰

The total daily urine was passed into a vessel containing a saturated solution of lead acetate. After complete precipitation, the lead acetate was removed by H_2S , the latter displaced and the urine concentrated by warming (under $45^\circ C.$) in a vacuum. The filtered solution was then shaken with 20 per cent. acetic ether for three hours, and thrice again with an excess of ether for three three-hour periods. The ether having been separated, the resultant fluid was made just more than slightly alkaline, naphthalin-sulpho-chloride added, and then the mixture was shaken for 12—24 hours, the alkalinity being maintained by frequent testing, and naphthalin-sulpho-chloride being added every three hours. After filtration, concentrated hydrochloric acid was added, and the precipitate obtained was shaken out with ether, and the ether washed with a small quantity of water. The ethereal solution was evaporated, either in a vacuum or by gentle heat, and the residue

dried and weighed before or after being crystallised from hot 20 per cent. alcohol. Or, after strong alkalisation, the amino-products were separated from the naphthalin-sulpho-amides by solution in dilute ammonia. The purified product was treated in the specified manner with barium chloride, the barium-naphthalin-sulpho-glycin separated and removed, the barium separated by the addition of hydrochloric acid, the resultant naphthalin-sulpho-glycin dried and weighed, and after crystallisation, the melting points of the crystals determined. The melting points varied from 151° to 155° C. In some instances, the esters were determined. In the glycocoll estimations, there was always a trace of amino-acids after the removal of the glycocoll, but with the quantities of the urine dealt with, it was too small to be treated other than qualitatively.

When shaking out the amino-acids with ether, at the junction of the ether and the mixture containing the acids, there was a mucoid-like brown scum which resisted solution.

GLYCOCOLL IN PATHOLOGICAL URINES

The following estimations have been made upon the urines obtained from children who were at the time in-patients of the Manchester Children's Hospital, and I am indebted to Dr. H. Ashby and Dr. H. R. Hutton for permission to utilise their cases, and to Dr. H. Rayner for the collection of the urines. The figures show variations from 0.05 to 0.25 gm. glycocoll during 24 hours.

CHILDREN

	Age	Disease	Glycocoll (24 hours)
J. B.	6 years	Mitral disease	0.0050
T. C.	7 "	Splenic anaemia	0.2510
J. W.	7 "	Anaemia (?)	0.0720
E. G.	8 "	Tuberculosis	0.0120
W. L.	$8\frac{3}{4}$ "	Pleurisy	0.0625
J. S.	10 "	Mitral disease	0.0600
R. H.	10 "	Chorea	0.0135
S. B.	10 "	Mitral disease	0.0060
F. F.	11 "	Persistent vomiting	0.0739
A. W.	12 "	Mitral disease	no trace
M. D.	13 "	" "	"
J. B.	13 "	" "	0.0865

In cardiac disease the quantities are infinitesimal and, in all probability, due to the possible errors of the method.⁹ Only in splenic anaemia is there any distinct elimination, and this is in a line with prior observations upon adults. In the cases of anaemia, pleurisy, and vomiting, however, there is certainly some excretion of glycocoll.

ADULTS

		Weight	Glycocoll per 24 hours
J. W.	Healthy	12 stones	0·0182 gm.
S. R.	Leuchaemia	9 „	0·1280 „
P. P.	Rh. arthritis	9 „	0·1168 „

Here again in leuchaemia and rheumatoid arthritis there is a marked excretion of glycocoll when compared with the amount excreted by a healthy person.

TOTAL NAPHTHOLIN-SULPHO-AMINO-ACIDS IN URINE

The following estimations show the limits and average of the excretion of the total monoamino-acids in the cases investigated.

	Date	Disease	Naphthalein-sulpho-ammo acid gm. per 24 hours
S. Mc.G.	1904	Gout	0·1022—0·5735
J. W.	„	„	0·2440—0·3598
J. W.	1905	„	0·0644—0·3350
Köln (1)	„	„	0·1050 in 500 c.c. of urine ; say 0·2500 in 24 hours
Köln (2)	„	„	0·1800 in 50 c.c. of urine ; say 0·3600 in 24 hours
J. F. S.	„	Diabetes	0·3500
W. W.	„	Normal	0·2785
W. W.	1906	„	0·1365

There is a certain similarity between all the quantities, but the gouty individuals exhibit a somewhat wide range of variation. The amount excreted in diabetes is more than a normal output, and this tends to confirm the recently published work of L. Mohr.

CASES OF GOUT DURING ACUTE ATTACKS AND DURING INTERVALS,
AND THE EFFECTS OF DIET, ETC.

T. W., male, *aet* 42. Long-standing case with pronounced gouty symptoms. Large tophi on all joints ; frequent discharges from tophi on phalanges. Amputation of one leg above knee for secondary suppuration in tophaceous joint.

Date		Naphthalin-sulpho-amino- acids per 24 hours		
1904.	Jan. 19	0·2810 gm.	Interval between acute attack	
"	" 22	0·2815 "	"	"
"	" 23	0·3598 "	"	"
1905.	Dec. 4	0·1024 "	Acute attack	Milk diet
"	" 5	0·1400 "	"	"
"	" 6	0·2234 "	"	"
"	" 7	0·1000 "	"	"
"	" 8	0·3350 "	"	"
"	" 9	0·0644 "	"	"

In this case an average excretion of amino-acids in the urine was maintained throughout long periods. During the acute attack, the amount was slightly less than that previously ascertained, but the daily elimination was irregular.

S. Mc.G., male. Chronic gout, numerous tophi, few acute attacks.

Date		Naphth-sulpho- amino acids for 24 hours		
1904.	Oct. 16	0·0870 gm.	Interval between acute attacks	Milk diet
"	" 20	0·0628 "	"	"
"	" 22	0·1850 "	"	"
"	" 26	0·1022 "	"	"
"	Nov. 18	0·1840 "	"	"
"	Oct. 15	0·4139 "	"	Meat diet
"	" 21	0·2630 "	"	Milk diet plus 50 gms. casein
"	" 23	0·5735 "	"	Milk diet plus 75 gms. casein
"	Nov. 15	1·6073 "	"	Milk diet plus 2 gms. glycocoll

In this instance, the addition of an excess of myo-albumins and of casein was associated with an increased amount of mono-amino acids in the urine, while the administration of glycocoll, which under ordinary conditions is almost entirely oxidised to urea, was followed by a marked increase in the output. The above figures are taken as averages from one experiment extending over 38 days. On several occasions the acids were estimated by Fischer's ester methods, the amounts varying from 0.0200 to 0.0700 gm. glycocoll ester.

The results of these determinations are almost all within the boundaries set forth by Embden and Reese as normal variations. The fallacies of the methods at present available may be responsible for the different findings, but there seems to be little doubt that small quantities of mono-amino acids are present in the urine, either free or combined with other substances. In the cases of gout here investigated, there appear certain variations in the amounts, and these differences are accentuated by feeding upon casein—which contains a large percentage of leucin and tyrosin—and after the administration of glycocoll. Of course these cases are insufficient for any possible interpretation of results. They are merely recorded as a contribution to the accumulation of facts and to indicate the necessity for continued observations. There is a little temptation to compare the figures obtained in conditions of altered and lowered metabolism, such as gout and leuchaemia, etc., with the appearance of glycocoll and tyrosin in the urine when large quantities of these substances are administered by the mouth, but at present there exists no basis for such comparison in the human subject.

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