Formation of urobilin / by Vaughan Harley.

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Publication/Creation

London : Printed at the office of the British Medical Association, 1896.

Persistent URL

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PAPER READ IN THE

SECTION OF PATHOLOGY AND BACTERIOLOGY,

At the Annual Meeting of the British Medical Association in Carlisle, July, 1896.

BY

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Reprinted for the Author from the BRITISH MEDICAL JOURNAL, October 3rd, 1896.

LONDON:

PRINTED AT THE OFFICE OF THE BRITISH MEDICAL ASSOCIATION,

429, STRAND, W.C.

1896.

FORMATION OF UROBILIN.

Since Jaffé [1] first demonstrated the appearance of urobilin in the urine of patients suffering from febrile diseases, its pathological significance has attracted the attention of numerous workers.

Urobilin icterus was described by Gerhardt [2] in 1878; in which the skin instead of having the greenish-yellow colour of ordinary jaundice has a dirty yellow colour, and the urine gives no reaction with Gmelin's test but shows a marked increase of urobilin. Although in normal urine the quantity of urobilin is extremely small, there is always present in it either some of it or its chromogen. The chromogen, which is called urobilinogen, is converted on standing by oxidation into urobilin. A urine which at first yields no distinct band of absorption in the spectrum may, after the addition of an acid, show the urobilin band at F from the chromogen having been oxidised into urobilin. The normal quantity of urobilin as estimated by G. Hoppe-Seyler [3] is from 0.8 to 0.15 grammes per kilo-weight in the twenty-four hours' urine. The late researches of Fr. Müller [4] show that from mere traces it may rise to as much as 0.20 grammes. The quantity is greatly increased in the urine in cases of liver disease and where there is absorption of large blood extravasations, as well as in diseases where there is great breaking down of the blood corpuscles, such as occurs in fever, scurvy, and pernicious anæmia.

As it was thought that urobilin was either derived from the blood or the bile pigments, various conjectures were made to account for its increase in quantity in the urine in certain diseases. Since it is thought that in certain pathological

[1] *Centralbl. f. med. Wissensch.*, 1868.
[2] Ueber Urobilinikterus. *Korresp. des Allg. artzl. Vereins in Thüringen*, 1878.
[3] *Virchow's Arch.*, v , 124, 1891, p. 34.
[4] Ueber Icterus. *Maly's Jahres. d. Thierchemie*, v., 22, 1892, p. 655.

conditions a diagnosis may be founded on the increase in the quantity of urobilin in the urine, a solution to the question of in what part of the organism it is formed is of very great importance, and the following is a brief summary of the theories that have been propounded up to the present.

1. *Hepatogenous Urobilin.*—The French are the chief supporters of this theory. They consider that instead of under normal conditions the liver cells forming bilirubin they form urobilin when they are diseased. The presence of an increase of urobilin in the urine they therefore consider arises from hepatic insufficiency.

2. *Hæmatogenous Urobilin.*—The supporters of this view consider the liver has nothing to do with the formation of urobilin, and that it is directly derived from the red blood corpuscles which are broken up in the circulating blood either by some chemical or toxic poison. Or it may be that the breaking up of the red corpuscles in large extravasations yield urobilin to the circulation, which, like any other foreign substance, is eliminated from the blood by the kidneys.

3. *Nephrotic Urobilin.*—Those who believe that the kidney is the source of urobilin consider that bile from any cause, after reaching the general circulation, is converted into urobilin during the process of its elimination by the renal epithelium, and then finds its way into the urine. According to the quantity of bile that is to be eliminated, all or only part of it is converted into urobilin, and this explains why urobilin may be increased when bile pigments are absent, or nearly so.

4. *Histogenic Urobilin.*—In the same way as in the last view the bile in the organism itself is believed to be the precursor of the urobilin. The bile pigment, after having reached the general circulation, and being deposited in the tissues as in ordinary jaundice, is slowly converted into urobilin, and the urobilin being more soluble than the bile, is then dissolved in the blood and is eliminated in the urine.

5. *Enterogenous Urobilin.*—Maly, who long ago showed that urobilin could be formed from bilirubin by reduction outside the body, considered that in the healthy organism the urobilin in the urine is derived from absorption into the blood from the intestinal tract, of the urobilin that has been formed from the bilirubin in its passage along the intestines. The late investigations of Müller and his pupil, D. Gerhardt, have added strong support to this last view. Müller found a

method of separating bilirubin from urobilin either in the urine or the fæces, and by means of the spectrophotometer he was able to estimate the amount of urobilin. He found that in obstruction of the ductus choledochus, from either calculi, morbid growths, etc., where no bile entered the intestines, urobilin entirely disappeared from both the fæces and the urine, while immediately after the obstruction to the duct was removed, and the intestines again contained bilirubin, urobilin reappeared in the fæces and urine. From this he considered that urobilin is formed from the bile pigments in the intestine, from thence absorbed into the circulation, and then excreted with the urine. The following experiment seems to prove this point:

A man with marked jaundice, whose fæces contained no traces of urobilin, was given daily by a stomach tube from 25 to 125 g. of pig's bile absolutely free from urobilin. On the second day after its administration urobilin appeared in the fæces, and on the third day it was detected in the urine. The administration of the bile being stopped, the urobilin disappeared from the fæces and urine.

Müller considered that the increase of urobilin during the absorption of large blood extravasations and in infectious diseases where blood destruction is going on is due to the blood pigment while passing through the liver being converted into bile pigment, and thus causing an excess of bile pigment, which in its turn gives rise to an increased formation of urobilin in the intestines and absorption of it from them. The result of Müller's experiment supports Maly's view that the urobilin met with in the urine is derived from the urobilin formed in the intestines by the reduction of bile pigment.

I will now adduce some experiments of my own on the formation of urobilin in the intestines, and show that the increase of urobilin in the intestines must be accompanied by an increase of intestinal bacteria to produce any marked increase of urobilin, and by increased intestinal putrefaction, without increase of bile, urobilin may be increased in the urine. Whether or not under certain pathological conditions it can also be formed in the tissues or blood I am not at present prepared to discuss.

Schmidt[5] showed that when a concentrated solution of perchloride of mercury is applied either to wet or dry fæces

[5] Schmidt, *Verhandlungen d. Congress. f. innere Medicin*, 1895, p. 320.

containing urobilin, in the space of a few minutes a bright rose-red colour is developed. The rose-coloured extract when separated and examined with the spectroscope shows the urobilin band between F and *b*.

In the presence of bile, in consequence of the conversion of bilirubin into biliverdin, the perchloride of mercury gives a bright green colour, so that this test is applicable to both substances. It is possible by this method to recognise very small quantities of urobilin. The colour is distinctly visible on a fragment of fæces after the treatment with perchloride of mercury when examined under the microscope, and until we had this method it was extremely difficult to recognise urobilin in small quantities in the fæces. On examining the different parts of the intestines and intestinal walls Schmidt found that the urobilin reaction yielded results differing not only in different parts of the intestine, but also in different cases.

In the small intestine he examined twenty-five cases in all, and found in one-third of them a faint red coloration in the upper part of the jejunum, both in the contents and in the wall. Most of the cases were children.

In another third of the cases examined the small intestine showed no red colouration whatever, or at the most only a faint pink colour towards the lower end of the ileum. In them the ileo-cæcal valve was strongly demarcated by the deep colour of the caecum and the faint colour of the ileum. In the other third of the cases the colour stood between the two first mentioned.

With regard to the large intestine, the upper part, close under the cæcum and in a few cases also the vermiform appendix, gave a deep pink coloration with the perchloride of mercury. This was less and less pronounced in the ascending transverse and descending colon. The red coloration of the walls slowly faded away until it entirely disappeared towards the sigmoid flexure. The contents of the large intestine, on the other hand, yielded a distinct pink colour as far as the anus.

The results I have obtained in the human subject are very similar. The contents of the various parts of the intestines were placed in small porcelain capsules and treated with the perchloride of mercury. The intestines themselves were likewise treated with the reagent after being well washed. The results obtained in this way led me to suppose that the urobilin reac-

tion of the small intestines is not so well marked in the case of adults as Schmidt would lead one to suppose from what he obtained in the case of children. I found it to be very exceptional to find any urobilin coloration in the contents above the ileo-cæcal valve.

Another interesting fact I noted which Schmidt drew no attention to is that the green or yellow colour due to the staining of the contents by bile in the duodenum and upper third of the jejunum tends to disappear about the middle third of the small intestine, where the contents become of a more or less greyish white; the green colour reappearing in the lower third of the small intestine as the contents of the ileum are always more or less coloured green with a slight tinge of brown. It would appear as if the bile in its passage along the intestinal canal is converted in the middle third of the small intestine into some colourless chromogen, to be reconverted into coloured bile lower down. For one can hardly suppose that all the bile pigment is absorbed from the upper part of the intestines in order to be re-excreted from the blood into the lower third of the small intestine.[6]

The urobilin coloration is only obtained when one reaches below the point in which the bilirubin has reappeared. A great part of the urobilin present in the intestinal contents is probably in the form of the chromogen urobilinogen, for from the results of analysis I find that larger quantities of urobilin are always to be obtained in the intestinal contents after they have stood a little while. This does not, however, entirely support the chromogen view of the case, as during the standing process after removal from the body the bacteria have a longer time to convert more bile pigment into urobilin.

In the case of a 12 days old child I had the opportunity of examining, through the kindness of Mr. Curtis, there was neither in the contents nor in the walls any nrobilin obtainable. This result agrees with that of Schmidt, who could get no reaction in the case of a 5 days old child.

Müller found no urobilin in the urine of newborn children, and none appeared in either the fæces or the urine until three days after birth. In the case of the 12 days old child above mentioned, the descending colon, sigmoid flexure, and rectum contained only a little colourless mucus, and the cæcum some greyish mucus but no urobilin. The upper part

[6] This colour change is not present in all cases and is, as a rule, most marked in dogs.

of the small intestine was bile-stained, and the middle third —as in the case of the adults—was almost colourless. The bile colour, however, reappeared in the ileum.

The staining of the wall of the cæcum with urobilin is supposed to be partly due to *post-mortem* diffusion, and partly to an absorption of urobilin taking place around it.

The results I obtained in the other animals in which examination was made immediately after death led me to a contrary conclusion, and I consider the staining of the intestinal wall was not due to absorption, or any indication of absorption, but merely a *post-mortem* diffusion, for in none of the animals—such as cats, dogs, and monkeys—did I obtain any staining of the walls of the intestinal tract, as I did in those of the human body I examined some hours after death. When the *post-mortem* examination is delayed, one always obtains more or less marked staining in the case of the animals, as in man. Schmidt says he found the same staining occurs in pigs as in man, but does not state how long after death they were examined. The animals I experimented on were always treated in exactly the same manner, and were killed with chloroform, the ligatures being always placed round the different parts of the intestines before the bowels were removed from the body. The contents of each part were separately placed in small capsules, the perchloride of mercury solution added, and the appearance they presented at various times noted. The intestines themselves were always well washed to remove any adherent contents before being treated.

In the case of the dogs I found, as in man, the middle of the small intestine, as a rule, colourless. I will here give the results obtained in one case as a type of what was found in the others:

The contents of the duodenum immediately below the pylorus were colourless, but the bile staining extended somewhat above the entrance of the bile duct into the duodenum, and downwards from it the yellow colour of the contents in the upper third of the intestines slowly faded away till the contents of the middle third of the small intestine were merely of a greyish-white hue. The contents of the lower third of the small intestine again became slightly greenish, and retained more or less of a greenish colour, somewhat tinged with brown, until just above the cæcum, at which point there was a suspicion of a fæcal odour and tint. Immediately below the ileo-cæcal valve the green colour of the

contents disappeared, and both in look and smell they resembled dog's ordinary fæces. After standing the perchloride of mercury yielded the bright green of biliverdin in the upper and lower thirds of the small intestine, where the yellow colour of bilirubin was originally found, but no green coloration was obtained in the middle third. Just below the ileo-cæcal valve the contents of the intestine gave with perchloride of mercury a marked urobilin reaction, and this continued to be the case as far down as the rectum. No colour reaction could be obtained in any part of the wall of the small intestine.

The dog now referred to had been fed on biscuit alone, with a little meat some twelve hours previously.

In the case in which the dogs were fed solely on meat, the colour met with in the middle third of the small intestines was more grey than white, and hæmatin could be chemically recognised in it. The same remark likewise holds good for cats.

In neither the normal dog nor cat have I been able to obtain in the intestinal contents much above the cæcum any urobilin, and when examined while in a fresh condition the walls of the small intestines throughout showed no staining with urobilin whatever.

In a monkey I examined I found the same changes occur as I found in man. The urobilin was found present in the intestinal contents from below the cæcum to the anus, but there was no trace of a pink coloration in the walls of the cæcum, colon, or rectum.

The results obtained in the foregoing experiments led me to investigate the formation of urobilin in two dogs from which the large intestine and cæcum had been removed four months previously. The small intestine having been artificially joined to the rectum, at the *post-mortem* examination the junction was found to be in one of the dogs (A.) 4 centimetres, and in another (B.) 6 centimetres above the anus. Both of these dogs, in spite of their having neither large intestine nor cæcum during the latter months, partook heartily of a mixed diet of meat and biscuits, and appeared in the best of health. The bile and urobilin in the fæces was examined by the method of Gerhardt.[7] The sulphates were estimated by Baumann's and the nitrogen by Kjeldahl's method.

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[7] D. Gerhardt, Ueber Hydrobilirubin und seine Beziehungen zum Ikterus, *Inaug. Diss.*, Berlin, 1889.

In dog A., where the large intestine had been removed, the fæces appeared to the naked eye to contain large quantities of bile, and bile was always chemically found to be present in large quantities, and though urobilin was fairly present on some days, as a rule it was only found in small quantities.

In dog B., on the other hand, the fæces never had a bilious appearance, and analysis always showed either that bile was entirely absent or present only in very small quantities, while urobilin again was always present in abundance. These two animals therefore gave diametrically opposite results, and consequently the results yielded by the aromatic sulphates, together with what was found at the *post-mortem* examination, are of great interest; for by the estimation of the quantity of aromatic sulphates in the urine we are able to recognise the amount of intestinal putrefaction that is going on; and in dog A., as was found in other dogs without the large intestine, this was markedly diminished.

Table showing the Nitrogen and Sulphate Output by the Urine in the Dogs after removal of their large Intestines. (Both Animals received daily 100 grammes of Lean Meat along with 100 grammes of Biscuit.)

	Days.	Weight of Dog in Kilos.	Total Nitrogen in Grammes.	Urine Sulphates.			
				Total.	Alkaline.	Aromatic.	Ratio.
					A.	B.	A. : B.
Dog A. ...	1	4·05	4.427	0.619	0.588	0.032	18 : 1
	2	4.05	4.815	0.637	0.640	0.033	19 : 1
	3	4.05	4.120	0.555	0.518	0.037	14 : 1
Dog B. ...	1	5·30	4.508	0.509	0.449	0.059	7 : 1
	2	5·30	4.110	0.391	0.342	0.049	7 : 1
	3	5·30	4.461	0.416	0.364	0.051	7 : 1

In the above table are only given the results of three days' analysis in each of the dogs as a type; since the results on other days during which the experiments were continued were in each case similar. In the above two dogs both received exactly the same diet, so that in reality dog B. was receiving less food than dog A. per kilo. weight. However, the difference was small. In normal dogs fed on exactly the same diet the ratio of alkaline to aromatic sulphates I have frequently found to be from 9 to 1 to 8 to 1. In dog A.,

instead of the ratio of alkaline to aromatic sulphates being as normal, they were from 19 to 1 to 14 to 1, showing a marked decrease in the aromatic sulphates, and therefore pointing to decrease in the intestinal putrefaction. These results I have noticed in other dogs in which the large intestine has been removed. In dog B., although on exactly the same diet, the ratio of alkaline to aromatic sulphates was 7 to 1, pointing to an increased intestinal putrefaction.[8] The cause of the intestinal putrefaction I am unable to give, but the dog was peculiar in many respects in the way its metabolism behaved, and was continually going off its food, although during the experiments above quoted it was on nitrogen equilibrium. As already stated dog A. passed in the fæces only very little urobilin, while in dog B. almost the entire, or the entire bile, was converted into urobilin. Both dogs were killed, and the contents of the alimentary tract, as well as the walls of the intestine, were treated in exactly the same way as the normal animals above described. In dog A., below the entrance of the bile duct, the contents were bile stained, which lost their colour in the middle third of the small intestine. The contents remained colourless until just upon reaching the rectum, when they assumed a brownish-green colour, and just below the junction of the small intestine with the rectum, 4 cm. from the anus, they still retained a brownish-green colour with perchloride of mercury. No urobilin was present in the small intestine, and a very doubtful reaction in the contents of the rectum. The walls of the intestine throughout did not give any reaction with perchloride of mercury.

In dog B., in the first third of the small intestine the contents were green; the middle third was empty; the lower third showed green-coloured contents at its commencement —that is to say, about 70 cm. from the anus—and urobilin was present in the contents at 40 cm. from the anus. The contents of the rectum—6 cm.—contained large quantities of urobilin and a small quantity of biliverdin.

The fact that the analysis of the fæces of dog A. showed more urobilin than was found in the contents at the *post-mortem* examination would be explained by the bacteria having longer time to act in reducing the bile pigment present.

Dog B., although found to be in sufficiently good health to gain in weight when properly fed, always had an excess of aromatic sulphates in its urine, and therefore increased

[8] The urine on standing deposited indigo blue as a precipitate.

intestinal putrefaction. In consequence of the increased intestinal putrefaction which extended up the small intestine, urobilin was formed even in the small intestine, and so much so that nearly all the bile pigment was converted into urobilin before it left the intestines.

The result obtained in this dog explains how in some human cases where there is increased intestinal putrefaction extending up the small intestine urobilin is formed high up in the small intestine instead of as, under normal circumstances, commencing below the cæcum. The increased urobilin in the urine would thus seem not only to be a pathological sign of increased bile in the intestinal tract, as pointed out by Müller, but also significant of increased putrefaction in the intestine.

That intestinal bacteria are capable of converting bilirubin into urobilin has been long known, and is mentioned by Schmidt. In confirmation of the above results calomel gives us strong support. It is known that calomel, when given by the mouth, causes the fæces to contain large quantities of bile pigment, and it has generally been put down as significant of the increased flow of bile into the intestine and increased peristalsis along the intestinal tract.

I have found in man that when small doses—$\frac{1}{2}$ gr. or so—of calomel are given at frequent intervals, the stools by degrees assume a green colour and contain large quantities of bile pigment and only small quantities of urobilin, and in these cases the calomel causes no purging. As we know, calomel diminished the aromatic sulphates in the urine, and therefore diminished the intestinal putrefaction. The green stool of calomel is in all probability partly due to increased bile, but chiefly to the diminution of intestinal bacteria, the biliverdin in consequence not being converted into urobilin.

Still further in support of the view that the increase of urobilin occurs in cases of increased intestinal putrefaction, I have found in one case since I obtained the above results, where there was a marked increase of aromatic sulphates there was also a great increase of urobilin. In fevers which are almost always accompanied by an increase of urobilin in the urine there is also an increase in the intestinal putrefaction; the same holds good for liver disease. In partial obstruction of the bile ducts the increased urobilin cannot be explained by increase of bile in the intestines, but seems to be the re-

sult of increased intestinal putrefaction. It would appear that the increase of urobilin in the urine is therefore not only of assistance in the diagnosis of diseases in which an increased destruction of blood corpuscles occurs, as stated by Müller and others, but also has significance in pointing to increased intestinal putrefaction.

In the case of dogs this does not hold good, for in none either examined by myself or by Dr. Putnam while working in my laboratory have we been able to discover any urobilin in the urine by any of the methods at present employed for its isolation or recognition. That dog's urine contains no urobilin has been already pointed out by A. Beck[9] and Müller.

Conclusions.

1. That bile pigment present in the upper part of the small intestine during its passage along the alimentary canal is converted into some colourless chromogen, to be again converted in the lower part of the small intestine into bile pigment.

2. Urobilin is, as a rule, formed in the large intestine below the ileo-cæcal valve, and only rarely in the small intestine; that is to say, only in those parts where intestinal putrefaction is most active.

3. The staining of the wall of the cæcum and large intestine with urobilin is due to *post-mortem* diffusion, and is not any indication of the absorption of urobilin in the living animal. Why it should in some cases be most marked in the cæcum and just below the cæcum, and not in the rectum, can only be explained by the fact that those parts are generally found in the *post-mortem* room more decomposed than the rectum.

4. The increase of urobilin in the urine, as well as having pathological significance—as has been already recognised in cases of internal hæmorrhages, such as cerebral, peritoneal, or hæmorrhagic infarctions and extrauterine pregnancy, and probably when red blood corpuscles are being destroyed, as in infectious fevers, scurvy, and pernicious anæmia—points also in favour of increased intestinal putrefaction, and may be a useful chemical test for such purpose.

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[9] T. A. Beck, *Wien. klin. Woch.*, August, 1895, p. 617.