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ANALYSIS OF A BILIARY CONCRETION; AND ON A NEW METHOD OF PREPARING BILIVERDIN.

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THE nature and composition of the various products contained in bile, or resulting from its decomposition, have, during the last twenty-five years, given rise to so much discussion, that any facts which may help to throw light on this subject cannot fail, I believe, to be serviceable to science. I have, therefore, the honour of communicating to the Chemical Society the present account of the properties and analysis of a peculiar biliary concretion, as an introduction to an investigation of biliverdin with which I am now occupied.

This concretion was extracted from the liver of a pig a few months ago by Mr. James Forrest, F.C.S., who kindly forwarded the specimen to me for examination. It was of considerable size, being about three inches in length, and two inches wide, and was entirely surrounded by a thin membrane, which isolated it from the tissue of the liver. Its substance is heavier than water, and has a compact structure, without any trace of crystallisation, more or less concentric, of a waxy appearance, and of a yellowish colour, which is neither bright nor uniform, until the substance is pulverised, when it appears as a brilliant yellow powder, similar to chromate of lead. This powder was found to be very slightly acid to blue litmus paper, and very little acted upon by water, alcohol, ether, benzol, and various other solvents, hot or cold. It is not volatile without decomposition, but melts and swells up, and afterwards takes fire, burning with a long fuliginous flame, *exhaling an odour identical with that which is produced in the same circumstances by certain resins, or when common varnish is burnt.* Concentrated sulphuric acid colours it first red and crimson, and then dissolves it with a magnificent emerald-green colour. From this solution water precipitated a green resinous substance, which was found to be biliverdin. Ether extracted from the substance of the concretion a little grease and some cholesterin,

which was identified after being crystallised from boiling alcohol, which yielded it on cooling in its characteristic pearly lamellæ, taking a fine purple colour with concentrated sulphuric acid. After the action of ether, alcohol extracted a notable amount of hyocholate of soda, together with a little free hyocholic acid, and a neutral white substance, which is neither soluble in water, acids, nor alkalis (hyocholin). The hyocholate of soda is isolated from this mixture by water, and upon evaporation the salt is obtained with its characteristic bitter taste, exceedingly permanent; and the free hyocholic acid is next obtained by a dilute solution of soda, when the third substance, which I call hyocholin, remains on the filter. The hyocholate of soda being decomposed by dilute sulphuric acid, yields the acid, which melts readily in hot water below 100° C, and I find that when hyocholic acid is thus melted it is no longer easily soluble in dilute ammonia.

When the calculus is treated with a hot solution of soda for a little time, a large amount of the yellow colouring matter is dissolved, and with it some caprylic acid, easily recognizable by its peculiar odour when the base is saturated with sulphuric acid, as well as by the odour of pine apple, which it gives on being digested with alcohol and hydrochloric acid. The greater portion of the concretion is formed of cholepyrrhin (or biliphein of Heintz) and mucus. The separation of these two substances can only be effected accurately by the following method:—

After determining the moisture at 105° C, the substance of the concretion is left in ether for 24 hours. The ether is then separated, and a new quantity added. In a day or two this is added to the former quantity, and the whole evaporated yields the cholesterin with a small amount of grease. The concretion is then treated several times with alcohol below boiling point, and the liquids being united and evaporated give the hyocholate of soda, with which is mixed some free hyocholic acid and the neutral substance (in appearance somewhat like palmitic acid) before alluded to. If sufficient ether and sufficient alcohol are used, and the whole is left for two or three days, a second treatment with these reagents is not necessary, provided the residue is washed with ether and alcohol. The calculus, being deprived of the substances soluble in these liquids, is next digested with alcohol strongly acidulated with hydro-

chloric acid, and the whole abandoned for a few days. The liquid soon becomes of a bright emerald-green colour, and the colouring matter is entirely dissolved as biliverdin, leaving the mucus, which can easily be separated by filtration. The green solution becomes more and more blue before the whole of the colouring matter is dissolved. When the liquid has become blueish-green it may be filtered, and the mucus washed with dilute alcohol previously acidulated. When water is added to the filtrate, biliverdin is precipitated as a pale bluish-green matter, but in so fine a state of division that it easily passes through a filter. The amount of biliverdin cannot be so well determined in this manner as by another method, to which I will allude presently, which may also serve to prepare biliverdin in large quantities; but it is the only way to determine the amount of mucus.

There is a considerable quantity of chloride of sodium present in the concretion, and also phosphate of lime, but *no trace of taurocholic acid or any other sulphur-compound could be detected in it.* Before proceeding further, the results of the analysis of this concretion may be stated thus :—

	I.	II.
Water	8·00	8·10
Cholesterin, with a small amount of grease	1·35	1·85
Mucus	11·50	10·98
Hyocholate of soda, with some hyocholic acid and hyocholine	2·75	2·75
Cholepyrrhin (Biliphein)	61·36	62·67
Carbonate of lime	1·55	
Phosphate of lime	3·25	
Soda	1·11	
Chloride of sodium	7·13	13·65
Caprylic acid, matters not determined, and loss in analysis	2·00	
	100·00	100·00

In its recent state the concretion contains 37 per cent. of water, but when pulverised and exposed to the air, it loses moisture until it contains only 8 per cent.

This kind of biliary concretion is occasionally met with, I believe, in the liver of the ox as well as in that of the pig. The yellow colouring matter, cholepyrrhrin (or biliphein),

which constitutes the principal portion of it, and the splendid green substance, biliverdin, which is obtained from it, are, without doubt, two of the most interesting organic compounds. The first is probably the same substance that exists in the bile, not only of man, but of all animals; and both present several points of resemblance with chlorophyll, or the green matter of leaves, and xanthophyll, or the yellow substance which takes the place of green chlorophyll in autumn. It has been asserted that the vegetable substance, chlorophyll, exists in certain inferior animals, such as *Hydra viridis* and several *infusoria*. It has also been remarked that chlorophyll has many analogies with the colouring matter of the blood, and, on the other hand, that biliverdin resembles chlorophyll, not only in its properties, but also by its composition. These considerations have led me to endeavour to ascertain whether chlorophyll and biliverdin are really identical in composition, and whether it is chlorophyll or biliverdin which has been found in the inferior animals alluded to. The results of this investigation I reserve for another paper. I will only state here that concretions, such as that which forms the subject of this paper, furnish us with a ready means of obtaining biliverdin in considerable quantities, and that the results of my examination of this substance, and of chlorophyll obtained from the ivy, lead me, so far, to the conclusion that biliverdin differs from chlorophyll only by the elements of two equivalents of carbonic acid.

In 1858 I observed (*Comptes rendus de l'Acad. Paris*) that the yellow colouring matter of leaves in autumn changed to a bright emerald-green in concentrated sulphuric acid. The yellow colouring matter of the concretion here described behaves in the same manner, and biliverdin may be easily prepared from it as follows:—The powder is digested in concentrated sulphuric acid at the ordinary temperature until the whole has become a dark emerald-green. It is necessary to add the substance to the acid in small quantities at a time, to avoid a rise of temperature, and to stir the mixture constantly. In a short time the mucus and other matters are destroyed or dissolved, and a dark green fluid is obtained. This is thrown into a large quantity of cold water, when the biliverdin is precipitated. It must be washed by decantation several times, and not upon a paper filter, which it clogs in a little time more or less completely. The product is finally dissolved in alcohol,

which leaves it quite pure on evaporation. If the quantity obtained is small, it is left as a hard green varnish, which will not allow water to permeate through it; it is quite devoid of crystallisation.

The substance designated as hyocholin, to which I may refer later, resembles palmitic acid, in some respects, but it does not dissolve in dilute alkalis, nor does it float upon water. It amounts to less than 1 per cent. of the concretion.

The mucus obtained as above has, when dry, the appearance of horn. It decrepitates and melts when heated, burning with an odour of burnt feathers. It swells up considerably in water when separated by soda, but alcohol deprives it of this property. Thus, when the concretion is boiled with soda-solution, some of the yellow colouring matter is dissolved, and so is a portion of the mucus, but the latter separates after a certain time, forming a voluminous jelly, which cannot be separated by filtration.

The solution of the cholepyrrhin in soda is bright yellow. When boiled for some time in contact with the air, it becomes green, and acids then precipitate from it *biliverdin mixed with mucus.*

The biliverdin prepared by concentrated sulphuric acid, as already described, dissolves entirely in alcohol with a magnificent green colour, which is very permanent. Even sulphurous acid and nascent hydrogen have no action upon it, after being left in contact with them for several hours, or even for days. On the contrary, the yellow substance from which it is derived has a great tendency to turn green, and is even affected by the action of light, which causes it to become much paler, and, in fact, after some time, nearly white. If biliverdin could be obtained in large quantities, it would be a most valuable tinctorial substance, equalled in brilliancy by no green colour hitherto produced.

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