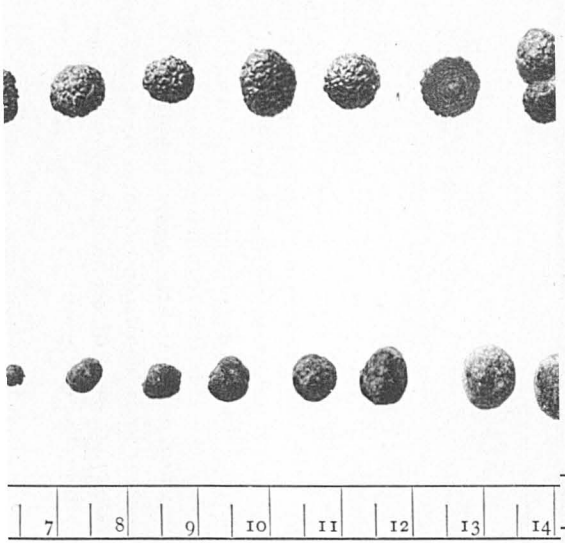
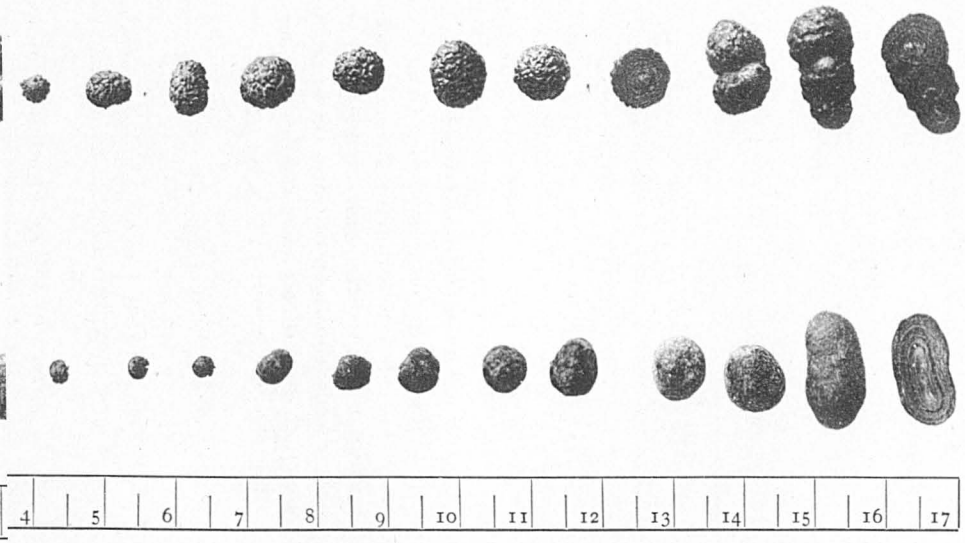


CALCULI FROM SQUETEAGUE.

one fish. A centimeter scale is introduced to show their size.

THE MERIDEN GRAVURE CO.



BILIARY CALCULI FROM SQUETEAGUE.

in each group are from one fish. A centimeter scale is introduced to show their size.

THE MERIDEN GRAVURE CO.

Contributions from the Biological Laboratory of the U. S. Fish Commission, Woods Hole, Massachusetts.

BILIARY CALCULI IN THE SQUETEAGUE.

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Pathological changes in marine animals seldom attract our attention, and it is indeed surprising that abnormal conditions are not more frequently found. The explanation of this must be either that these animals are remarkably free from disease or that the diseased individuals are quickly eliminated by the untoward conditions.

In 1901, while at the Fish Commission laboratory at Woods Hole, my attention was called to the presence of gallstones in the squeteague (*Cynoscion regalis*) by Mr. A. K. Krause, who had occasion to collect the bile from some three hundred specimens of this species during the summer. Of this number only four showed any lesions of the liver or bile passages. In one, the liver was apparently cirrhotic; from the other three, gallstones were collected and preserved for the following investigations.

The calculi in the first squeteague completely filled the gall bladder and the gall duct, in which no bile was found. These calculi (shown in the first or upper group) numbered 16 in all, weighed 2.465 grams, and were of all sizes from a big bean to a BB shot. The largest dried over sulphuric acid weighed 0.6725 grams; was 1.5 centimeters long, 0.5 centimeter thick, and 0.75 centimeter wide. All of these calculi were more or less nodulated, as can be readily seen from plate XXI. When bisected in a longitudinal plane they are found to consist of concentric layers arranged around one or more nuclei, which were very small and consisted chiefly of cholesterin. It being impossible to pulverize the air-dried stones, they were minced as fine as possible and boiled with water.

The aqueous extract (*a*) contained only a trace of either organic or inorganic matter (see p. 134). The solid residue (*b*) was extracted with hot alcohol. The alcohol (*c*) was decanted and evaporated, giving a mere trace of bile pigments, with a little cholesterin. The solid residue (*d*) was next digested with ether until there was no further extraction. The ether solution (*e*) on evaporation yielded fat and cholesterin. The latter crystallized in characteristic plates which were easily identified under the microscope. Dissolved in chloroform these crystals gave the characteristic cholesterin reaction after the addition of sulphuric acid. The solid residue (*f*) was now extracted with dilute hydrochloric acid (1:3) for 12 hours. Effervescence of CO_2 indicated the presence of carbonates, but in small amounts. The acid solution (*g*) was decanted, evaporated to dryness, and ignited. The ash dissolved readily in dilute hydrochloric acid and on analysis showed the presence of calcium, magnesium, iron,

phosphoric acid and sulphuric acid. The solid residue (*h*) from the HCl extract was washed with water and extracted several times with hot chloroform, which took out small quantities of bilirubin at each extraction (*i*). This method evidently did not remove all the pigment, so the solid residue (*j*) was extracted with hot alcohol containing a little HCl. This removed a pigment in considerable quantities (*k*) which gave the qualitative tests for bilirubin. The solid residue (*l*) was digested in ether for 12 hours, but nothing was extracted (*m*). The residue (*n*) gave strong reactions with Millon's reagent and with the xanthoproteic test. It was divided into two proportions. One portion (*o*) was oxidized with KNO_3 and Na_2CO_3 and tested for phosphoric and sulphuric acids. Both were positive. A control test with the reagents alone gave no reaction. The other portion (*p*) was boiled with dilute hydrochloric acid for two hours. The resulting solution was examined for reducing sugars, but with negative results. It would seem, then, from the above that the proteid substance was of the nature of a nucleo-albumin and not a mucin. (See Table I.)

The small amount of calculi in the first or upper group (plate XXI) not used in the preceding analysis was estimated quantitatively for the principal constituents, with the following results:

Calculi dried to constant weight over H_2SO_4.

	Per cent.
Cholesterin and fat	2.85
Mineral	3.65
Bilirubin	16.14
Nucleo-albumin	65.59
Water	11.52
Soluble in water	Trace.
	99.75
Total ash of calculi	4.32

The fat was in excess of the cholesterin, although a quantitative separation was not made.

The calculi in the second group (plate XXI) were from another squeteague (a male). They differed from the first lot in being smoother and less nodulated. When bisected longitudinally they exhibited the same concentric structure as the others. These stones were found not only in the gall bladder and gall ducts, but also in the intestines, and, strangely enough, were embedded in the tissues between the liver and intestines. No lesions or scars appeared either in the bile ducts or in the intestines, yet these stones must have broken through the walls of these passages at some earlier time. The gall bladder and duct contained a small quantity of bile.

There were in all eleven stones, weighing 1.865 grams. The largest dried over sulphuric acid weighed 0.645 grams, was 1.7 cm. long, 0.8 cm. wide, and 0.7 cm. thick. The qualitative analysis agreed with the preceding and was carried out in the following way: The dried substance, finely minced, was extracted with ether until nothing more was dissolved. The ether extract (1) on evaporation contained cholesterin and a small amount of fat (see p. 135). The residue (2) was digested with dilute (2 per cent) hydrochloric acid for 12 hours, giving a slight effervescence of CO_2. The extract (3) on evaporation showed but little residue and was united with (5) for further analysis. The residue (4) was now extracted four hours with warm dilute HCl, which removed most of the mineral matter. The extract (5) was analyzed directly for inorganic substances. This gave relatively large quantities of phosphoric acid,

calcium, and magnesium, smaller quantities of sulphuric acid, and a slight amount of iron. The residue (6) was extracted with hot chloroform, and the extract (7) upon evaporation gave but little bilirubin. The residue (8) was boiled with alcohol. The alcohol extract (9) when evaporated gave a small amount of bilirubin. Thinking that there might be yet a bilirubinate which had not been decomposed by the dilute HCl, the residue (10) was extracted with hot dilute HCl alcohol for three hours. The acid-alcohol extract (11) upon evaporation yielded a considerable quantity of pigment which had the properties of bilirubin. A portion of the residue (12) gave strong reactions with Millon's reagent and by the xanthoproteic test. The remainder was divided into two portions. The smaller (14) was fused with KNO_3 and $HNaCO_3$ in order to determine the presence of P and S in the organic molecule. The product dissolved in hot dilute nitric acid gave good reactions for phosphoric and sulphuric acids. The larger portion (13) was washed, dried to constant weight at 105°C., and the per cent of N estimated by the Dumas method. An accident at the close of the determination prevented an accurate estimation. There was at this time 13.+ per cent N. The estimation, being of necessity low, can not be taken as an absolute indication of the nature of the substance, yet it would seem to suggest a nucleo-albumin rather than a mucin. (See Table II.)

The quantitative analysis of these calculi dried to constant weight at 100° C. yielded:

	Per cent.
Cholesterin (and fat)	0.47
Bilirubin	22.39
Nucleo-albumin	70.69
Mineral	5.10
	98.65

The difference in per cent of fat and cholesterin in the two analyses is certainly striking. The amount of cholesterin in the two cases varied but little as far as could be determined by qualitative reactions, but the fat in the latter case was evidently very much less.

The gallstones in the third or lower group, taken from the third squeteague (a female), were much smaller than the preceding. The largest, dried over sulphuric acid, weighed 0.11 gram and the entire 24 weighed only 0.935 gram. The appearance, except size, did not differ from No. 2. The calculi were also found in the gall bladder, gall duct, intestines, and embedded in the tissues surrounding the intestines and liver. The gall bladder contained a quantity of bile. The qualitative analysis did not differ from the two preceding, and a quantitative determination was not made.

The three fish from which the above calculi were taken were apparently in normal condition as far as could be determined when taken from the water. The livers were perfectly normal in appearance. It is a difficult matter to collect the urine from these animals, as it is generally eliminated as soon as they are taken from the water; but the small quantity of urine that was collected showed the presence of no bile pigments. Observations of this character would certainly be of interest in the light of comparative physiology.

Perhaps the most interesting feature in the analyses described above is the high per cent of nucleo-albumin, differing in this respect from the gallstones reported from other animals. Further observations on the formation of these calculi might prove to be of value in explaining the causes of such deposits.

TABLE I.

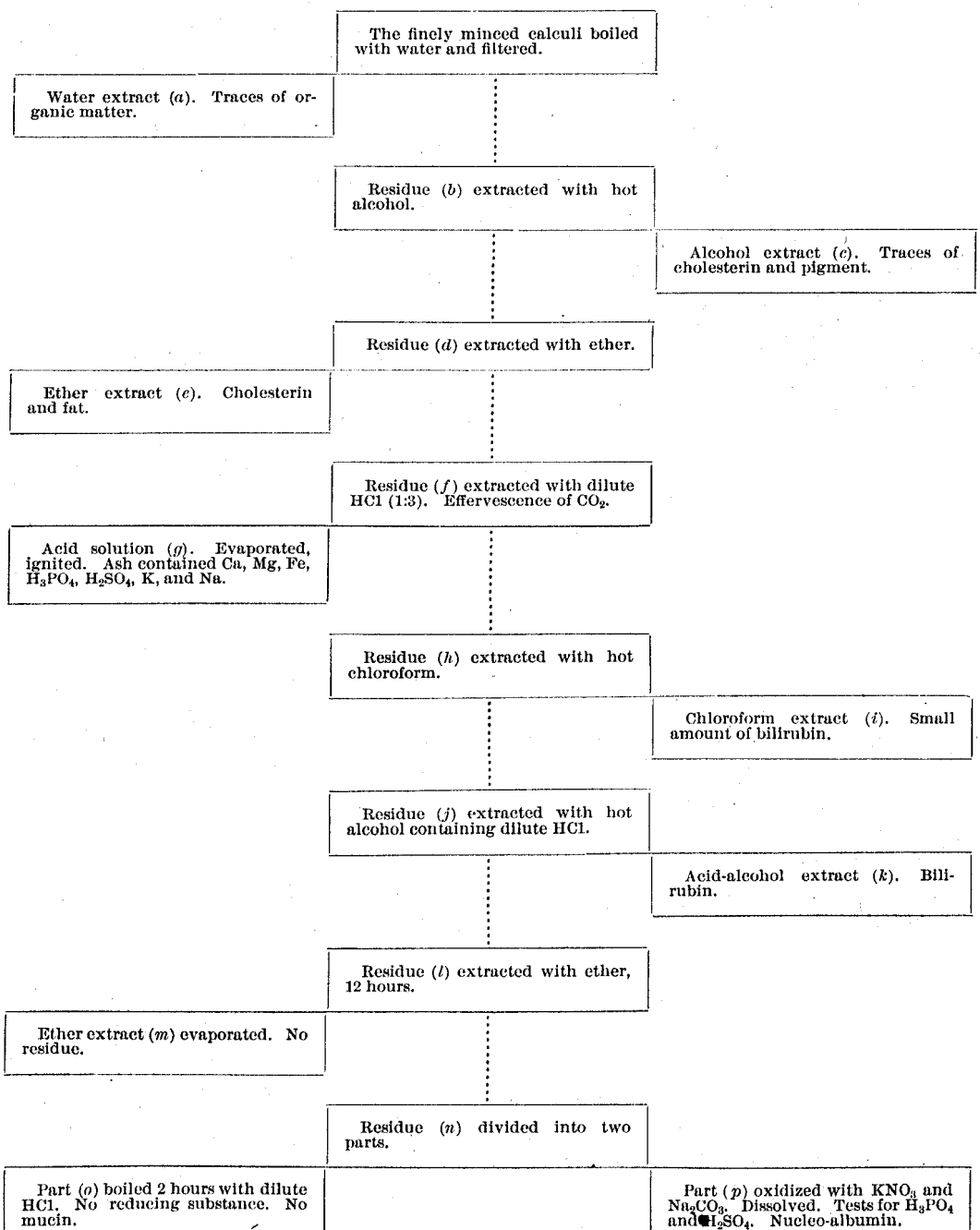
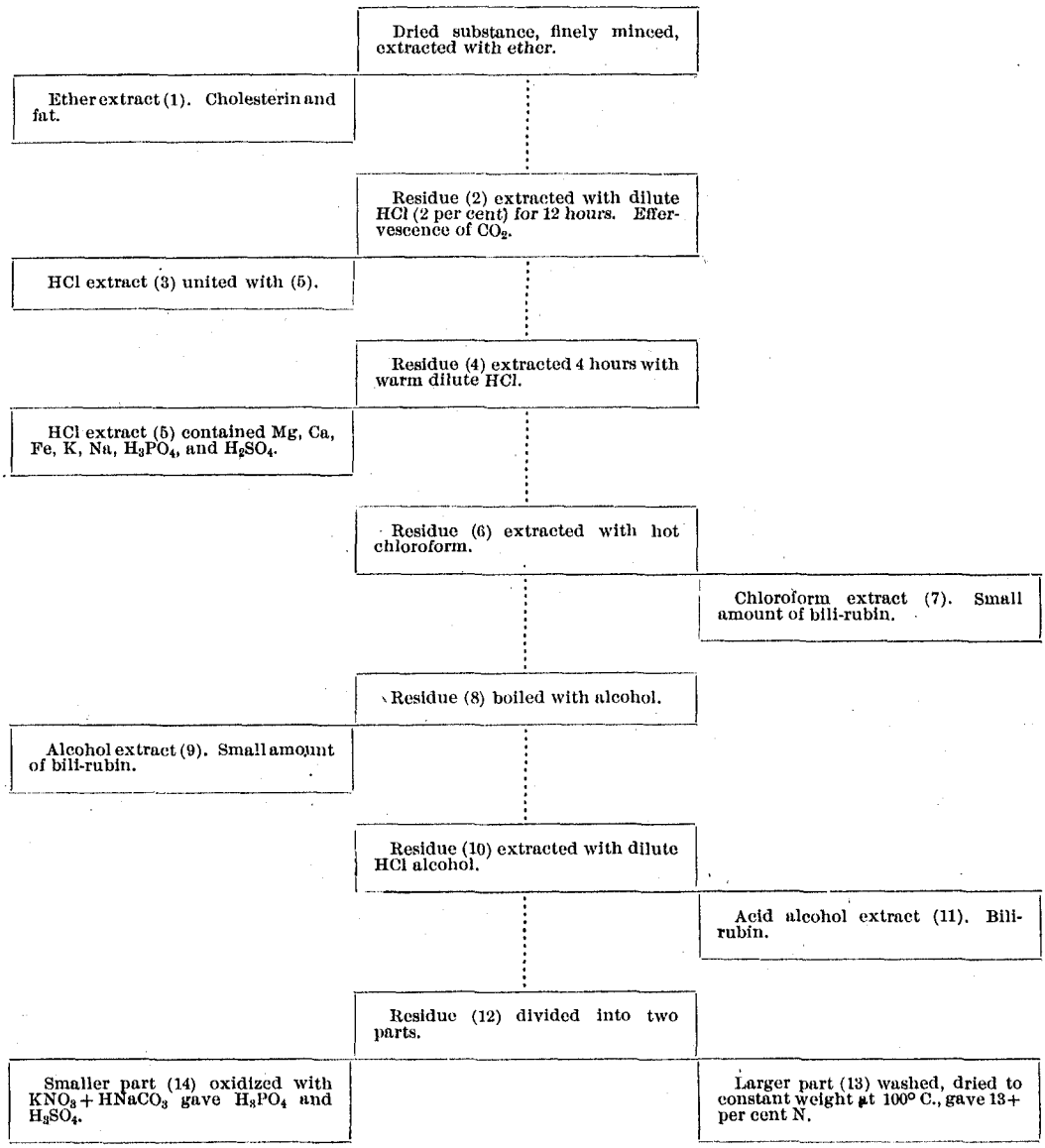

The finely minced calculi boiled with water and filtered.
Water extract (a). Traces of organic matter.
Residue (b) extracted with hot alcohol.
Alcohol extract (c). Traces of cholesterin and pigment.
Residue (d) extracted with ether.
Ether extract (e). Cholesterin and fat.
Residue (f) extracted with dilute HCl (1:3). Effervescence of CO_2.
Acid solution (g). Evaporated, ignited. Ash contained Ca, Mg, Fe, H_3PO_4, H_2SO_4, K, and Na.
Residue (h) extracted with hot chloroform.
Chloroform extract (i). Small amount of bilirubin.
Residue (j) extracted with hot alcohol containing dilute HCl.
Acid-alcohol extract (k). Bilirubin.
Residue (l) extracted with ether, 12 hours.
Ether extract (m) evaporated. No residue.
Residue (n) divided into two parts.
Part (o) boiled 2 hours with dilute HCl. No reducing substance. No mucin.
Part (p) oxidized with KNO_3 and Na_2CO_3. Dissolved. Tests for H_3PO_4 and H_2SO_4. Nucleo-albumin.

TABLE II.


Dried substance, finely minced, extracted with ether.
Ether extract (1). Cholesterin and fat.
Residue (2) extracted with dilute HCl (2 per cent) for 12 hours. Effervescence of CO_2.
HCl extract (3) united with (5).
Residue (4) extracted 4 hours with warm dilute HCl.
HCl extract (5) contained Mg, Ca, Fe, K, Na, H_3PO_4, and H_2SO_4.
Residue (6) extracted with hot chloroform.
Chloroform extract (7). Small amount of bili-rubin.
Residue (8) boiled with alcohol.
Alcohol extract (9). Small amount of bili-rubin.
Residue (10) extracted with dilute HCl alcohol.
Acid alcohol extract (11). Bili-rubin.
Residue (12) divided into two parts.
Smaller part (14) oxidized with $KNO_3 + HNaCO_3$ gave H_3PO_4 and H_2SO_4.
Larger part (13) washed, dried to constant weight at 100° C., gave 13+ per cent N.