# LXVI. CHANGES IN THE CHEMICAL COMPOSITION OF THE TISSUES OF THE HERRING IN RELATION TO AGE AND MATURITY.

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(*Received March 17th, 1924.*)

Numerous proximate analyses of the tissues of the herring and other clupeoid fishes have been published from time to time. Milroy [1906, 1907, 1908] and Johnstone [1915, 1918, 1919, 1920] have established the fact that herrings caught at different times and in different localities vary widely in composition, especially in fat-content, and they have correlated these variations with the reproductive phases and periods, and with the bodily measurements of the fish. Up to the present, however, no attempt has been made to ascertain the extent to which age, as distinct from maturity or size, affects the chemical composition of the tissues. This is doubtless due to the fact that no reliable and universally applicable criterion of age has been available. Length, and other standardised measurements, may afford fairly reliable evidence as to the relative ages of the individuals in a single shoal, but the existence, within the species, of separate "races" of fish, of widely differing characters, invalidates any comparison of data obtained from other and distant shoals.

It is now widely believed that the number and distribution of the so-called "winter-rings" upon the scales of the herring are a reliable guide to the age of the fish, and upon this basis the samples used in this investigation have been classified. (Since the methods of scale-reading are probably not widely known outside the circle of fishery research, a note descriptive of the process has been added at the end of the paper. It must be admitted that the validity of the method still lacks final proof; so far, however, the data obtained by tissue-analysis have been corroborative.)

During the fishing-season (June—September) of 1923, analyses were made of a series of herring samples taken from the Peel (Isle of Man) landings, each sample having been selected by Mr W. Birtwistle, of the Oceanography Dept. of the University of Liverpool, so as to be homogeneous as regards age and sexual maturity. This necessarily involved a scale-reading and a determination of the gonad-condition on the Hjort scale, upon each of the 156 fish

analysed. Mr Birtwistle's invaluable co-operation, most gratefully acknowledged, made it possible to examine samples of fish in their third, fourth and fifth years, and ranging through successive stages of gonad-development from maturing virgins to "matties" and "spents."

Briefly, the results of this part of the investigation may be summarised by saying that age, considered as a separate factor, appears to have much the same influence upon the chemical composition of the muscle-substance as sexual maturity, also separately considered. In other words, individuals in the same stage of gonad-development, but differing in age, show a sequence of water/fat ratio, and nitrogen-content, similar to that of individuals in successive stages of gonad-development but presumably of the same age. The older, and therefore larger, herrings, with full roe or milt, have a higher calorific and food-value than younger, even if mature fish.

Included in this paper, also, are the results of some analyses of herrings from the Clyde Area, made in 1922, by Mr John Secker, B.Sc., sometime Biochemical Assistant at the Millport Marine Station. For the use of these data, hitherto unpublished, I am indebted to Mr R. Elmhirst, F.L.S., and the Scottish Marine Biological Association, and personally to Mr Secker, for various explanatory notes. In carrying out these latter analyses, special attention was paid to the possible rôle of phosphorus in the tissue-changes involved in the sexual cycle. The results are of value, in that they establish further general data as to the variations in the proximate food-materials, but it is clear that much further and more detailed work will be necessary before it is possible to advance any general theory as to the part played by phosphorus and the lipins in the history of fat-metabolism and translocation.

Finally, my thanks are due to Prof. Jas. Johnstone, D.Sc., and Mr W. C. Smith, respectively Director and Curator of the Port Erin Station, for helpful advice and assistance.

## Analytical Methods.

[*The following descriptions apply only to the Port Erin samples, unless otherwise noted.*]

The number of fish taken for an analysis was generally determined by the supply available—each sample consisting of fish of a single age-group (as determined by scale-rings) and, so far as possible, in the same stage of maturity. This entailed much labour in the selection of the samples from the catch as landed, and occasionally very small numbers only were obtainable. A sample of ten fish was aimed at, but some few samples were as small as four, while one included 15 fish; the mean number per sample was eight. Samples were duplicated, from time to time, throughout the season. The fish were sent to Port Erin by rail from Peel, I.O.M., and in every case the weighed portions for analysis were being dried within twelve hours after the death of the fish.

*Preparation of samples for analysis.* The fish, forming a homogeneous sample, were wiped free from adherent slime and loose scales, and two parallel cuts were made, about 1 cm. apart, down the full length of the side of the fish, from "shoulder" to tail. The strip of skin, so isolated, was picked up by forceps at the shoulder of the fish, and stripped off, with its attached scales—this operation was perfectly easy, and exposed a long narrow strip of muscular tissue. The point of a dissecting-knife was then inserted between the strip and the backbone, and while the strip was raised by forceps, it was freed from the bone along its length. In this way strips were taken from each fish comprised in the sample—one strip from each if there were eight or more fish, or two strips, one from each side, if the sample was a small one. The strips were laid side by side on a clean plate, and then, by drawing a sharp knife transversely across them all, small blocks of tissue, each about 0·5 cc. in volume, were separated from the shoulder, middle, and tail regions of each strip; these were lifted by forceps, and dropped into a dried and weighed extraction thimble (22 × 80 mm.) supported in a dry tared weighing bottle. It is necessary to sample the tissue at different points along the length of the fish, as there is some evidence that the fat, at any rate, is unequally distributed throughout the muscles [Johnstone, 1918; Milroy, 1908]. In this way, a fair sample of the edible muscular portion of each fish was represented in the material analysed.

In the case of the Scottish samples, the sexes were analysed separately, but when dealing with the Irish Sea fish no distinction of sex was made, since previous work indicates that the difference between the muscle-substance of the sexes is small and inconstant.

The composite sample taken for analysis was, roughly, 10 g. in each case.

During the entire process, there was never any visible loss of oil from the tissue, even in the comparatively fat-rich summer samples. Johnstone [1918] emphasised the care necessary to prevent loss of oil during sampling, which he effected by removing from each fish a series of thin transverse slices. This method was followed in the case of the Scottish samples, but it is not thought that any differences between the analytical results are attributable to this cause. Milroy [1906, 1908] passed the whole of the muscular substance of each fish through a fine mincing machine, afterwards removing portions for analysis from the pulped mass; it seems hardly possible that some loss or, at any rate, separation, of oil should not have occurred during this process.

*Estimation of Water.* The extraction thimble, containing about 10 g. of wet tissue, prepared as above, and supported in a weighing bottle, was placed in a steam-oven, maintained at 96°–98°. Consecutive weighings were constant after about 18–20 hours at that temperature, though in practice, the samples were dried for at least 24 hours, before the two check-weighings, to the nearest mg., were made. It is not claimed that every trace of water was driven off under these conditions, but the method was convenient, and gave consistent results; in view of the comparatively large errors involved in sampling, it was

not thought justifiable to aim at the highest precision in this, or in the subsequent operations.

The error introduced by oxidation of fatty substances, at the temperature of the drying process, to which attention has been drawn by Atwater and Woods [1906], has been investigated by Johnstone [1918], who has shown that the resulting increase in weight need not be taken into consideration when the values sought are intended only for general or dietetic purposes. It will be seen, however, on reference to the following section, that the error which may be introduced by oxidation of the fats, at another stage of the analysis, is by no means negligible.

*Estimation of Fat.* The term "fat" is here used as synonymous with "carbon tetrachloride extract," in the comprehensive sense in which it is usually employed in food-analyses, since phosphatides and possibly other substances are extracted with the true fats, and weighed together with them. Milroy [1908] extracted the dried tissue, in Soxhlet's apparatus, first with alcohol, and afterwards with ether, but does not mention the amount, if any, of the alcoholic extract. The extractions of both the Port Erin and Scottish samples, following Johnstone's method [1915, 1918], were made with pure carbon tetrachloride (B.P. 76°·7). The thimble, lightly plugged with glass-wool, was placed in a 100 cc. Soxhlet extractor; 150 cc. of carbon tetrachloride were placed in the receiving flask, a small portion being used to rinse traces of adherent oil from the weighing bottle. Extraction was allowed to proceed in the usual way for about two hours, with the siphon operating every five minutes. The extracting solvent was quite colourless after the third or fourth siphoning, even in those cases where the first extract was dark brown.

After extraction, the bulk of the solvent was recovered by distillation, and the flask containing the dark oily residue was placed in the steam-oven and dried to constant weight, this requiring as a rule about 6–8 hours. As a check, the thimble, with its fat-free contents, was also dried to constant weight, and the loss of fat ascertained by difference. As a result, it was found that the weight of extracted fat was invariably greater than the loss sustained by the dried tissue, the mean difference, calculated on 18 samples of the Port Erin series, amounting to 1·96 % of the absolute weight of fat present. It should be noted, however, that the fat-content of the original tissue, calculated on the basis of the carbon tetrachloride extract, exceeds only by 0·3 % that calculated upon the mean loss in weight of the extracted tissue. The latter value is believed to be the more reliable, and is that reported in the analytical tables.

Since the drying was most carefully conducted, and final constancy of weight established, there can hardly have been any appreciable retention of carbon tetrachloride by the oily residue, and the difference must be largely, if not wholly, accounted for by oxidation of the free fat. The stage at which such oxidation occurs, however, is not clear, for when control experiments were made, drying the extracted oil in a slow stream of $CO_2$ led in through

the tubulure of the steam-oven, no improvement was noted beyond a slight speeding-up of the drying process. The precaution was always taken, two or three times during the period of drying, and before weighing, to blow the heavy carbon tetrachloride vapour out of the flask by a jet of air from a foot-bellows. A quantity of the carbon tetrachloride, as used for the extractions, was boiled for some hours under a reflux condenser, then distilled off, and the flask dried in the oven and weighed, exactly as in the estimations—there was no ponderable residue.

*Estimation of Protein.* The usual Kjeldahl method was followed, with a few slight modifications dictated by convenience. In a 250 cc. Kjeldahl flask were placed 0·5 g. of the powdered, dry, fat-free material; 10 g. of potassium sulphate, and 20 cc. of pure conc. sulphuric acid; to accelerate oxidation, 0·3 g. of crystallised copper sulphate was added. After boiling gently for 2–2½ hours, the then clear solution was cooled, and diluted with 50 cc. of distilled water. The flask was provided with a rubber stopper, tap-funnel and splash-head, the latter connecting up with a vertical condenser, of which the lower end dipped below the surface of 75 cc. of $N/10$ sulphuric acid, containing a few drops of methyl red. A fragment of zinc was dropped into the dilute acid mixture in the flask, then, immediately connecting up, an ample excess (100 cc.) of 40 % caustic soda solution was rapidly run in through the tap-funnel. The alkaline mixture was boiled for at least 30 minutes, no trouble from "bumping" being experienced. The back-titration was made with $N/10$ NaOH, the end-point being quite sharp. As a rule, about 50 cc. of the acid were neutralised by the evolved ammonia, each cc. corresponding to 0·0014 g. of "total nitrogen."

There is some uncertainty as to the applicability of the usual factor ($\times$ 6·25) when converting total nitrogen into *fish* protein. This is discussed by Atwater [1892] and Johnstone [1918], the latter inclining to the view that the estimation of protein by difference—*i.e.* assuming that whatever is not water, nor carbon tetrachloride extract, nor non-volatile mineral matter ("ash"), is protein—is good enough for general and dietetic purposes. It is not, however, accurate enough for metabolic studies, in which the variations in the amount of nitrogenous but non-protein substances, throughout the seasonal and sexual cycle, require to be established. It is quite certain, moreover, that a small though variable proportion of the nitrogenous substances present in the fresh tissue is carried through with the fats during the carbon tetrachloride extraction, in the form of phosphatides (lecithin, etc.). To ascertain the possible magnitude of this source of error, Kjeldahl determinations were made upon four samples of fish from the Clyde area, using, in each case, the fresh moist material and the dry, extracted residue. Expressed in both cases as a percentage of the original wet weight, the protein ($N \times 6{\cdot}25$) in the former is 1·5 % greater than that in the latter. It may be noted, in this connection, that Johnstone [1919], while examining canned herrings, found that the carbon tetrachloride extracts contained from 1·0–1·62 % of "proteid."

It would seem, therefore, very desirable that the Kjeldahl estimations should always be made upon the fresh tissue; owing, however, to the necessity of dealing rapidly with the samples as obtained, it was usually found impossible to do this, and the dried, fat-free material was set aside for nitrogen-determination at a later date. Until such time, therefore, as it is possible to determine the nitrogen-content of every carbon tetrachloride extract, and to make the necessary correction, the protein-figure must be accepted with the reservation that it is somewhat too low, from this cause, and that in any case, the 6·25 factor may not be strictly correct. That both causes operate in the same direction may be seen from the variations in the percentage of nitrogen calculated upon the dry, fat-free, ash-free tissue-residue; these values are appended to the table of analyses (Table II), in the column "N." They are invariably less than 16, which is the mean percentage assumed when the factor × 6·25 is used. The mean value of "N," for 17 samples, is 14·5, whence the appropriate Kjeldahl factor is 100/14·5 = 6·9. The extent to which the values of "N" diverge from the standard figure is an indication both of the varying nature of the tissue-proteins and of the amount of nitrogenous but non-protein matter which goes to make up the total of the dry, extracted, and ash-free muscle substance.

*Estimation of "Coagulable Protein."* Estimations of the coagulable protein were made only upon the muscular tissue of the Scottish samples. The method followed was that of Milroy [1907]. 5 g. of the composite sample of fresh muscle were ground in a mortar with 5 g. of anhydrous sodium sulphate; 100 cc. of methylated spirit were added, and the mixture boiled under a reflux condenser for three-quarters of an hour. The spirit was then decanted off, and the coagulated residue washed with successive quantities of boiling water, until the washings were free from sulphate. It was then submitted to the usual Kjeldahl estimation.

*Estimation of non-volatile Mineral Matter.* This was determined by igniting about 0·5 g. of the dry, fat-free residue in a small porcelain dish. After heating to dull redness for some time, a few drops of conc. nitric acid were added to the dark ash, which became white on re-heating. It was impossible, of course, to avoid including a few small bones, when sampling the herring flesh, and these tend to raise the ash-content; again, a certain, if small, proportion of the mineral matter consists of chlorides and other volatile salts—these are lost to the analysis; finally, a trace of phosphorus in organic combination may be carried away during the estimation of the fats. Consequently, the values in the ash-columns represent the amount of non-volatile inorganic matter in the fat-free substance; it is probable, however, that they approximate fairly closely to the true "ash," or mineral-content of the living tissue.

Ash determinations were made only upon the Port Erin samples.

*Estimation of Phosphorus.* The phosphorus-content was determined, in the case of the Scottish samples only, upon the muscle, gonad and liver, of each of the sexes. Neumann's method, slightly modified, was employed. After

oxidation with conc. sulphuric acid, as in the first stage of the Kjeldahl nitrogen estimation, the mixture was cooled and diluted, nitric acid was added, and 20 cc. of 80 % ammonium nitrate, after which it was heated to about 85°, when excess (about 20 cc.) of 10 % ammonium molybdate was added. After standing for 15 minutes, the ammonium phosphomolybdate precipitate was filtered off through a wad of filter-pulp, using suction; both flask and precipitate were then washed free from acid. The precipitate was returned to the flask, and dissolved in a known excess of 0·5 *N* NaOH. The solution was boiled to remove ammonia, and the excess of alkali titrated with 0·5 *N* $H_2SO_4$, using phenolphthalein as indicator. Each cc. of 0·5 *N* alkali used to dissolve the precipitate corresponds to 1·268 mg. $P_2O_5$.

As in the case of the other estimations, the validity of the figure obtained is subject to certain reservations. The phosphatides extracted with the fats, containing as they do about 4 % of their weight of phosphorus, account for a certain, if small, loss of this element, while inorganic phosphates are known to be somewhat soluble in the usual fat-solvents when these are not perfectly anhydrous. Even if it were possible to use and maintain the solvent in a perfectly anhydrous condition, it is very difficult to ensure that all traces of water have been removed from the tissue. The total $P_2O_5$-content is small, amounting, on the average, to 0·56 % of the wet weight of the muscular tissue. The extent to which the organically combined phosphorus is removable by carbon tetrachloride or other fat-solvent may be judged from a series of seven determinations, which show that, on the average, the $P_2O_5$-content of the extracted tissue is 26 % less than that of the fresh material, both values being referred to the original wet weight.

*Note on Carbohydrate.* No estimations of carbohydrate have been made, either on the Port Erin or on the Scottish samples, since the amount present in the tissues of the herring (and in fact of most sea-fishes), whether as glycogen or in some other form, appears to be negligibly small at all seasons.

Stirling [1884] was able to demonstrate but the merest trace of reducing sugars in the livers of "mattie" or filling herrings, and showed that this condition was independent of food-intake. Milroy [1908], while believing that glycogen might vary in amount, along with other substances, during the various stages of reproductive activity, found that the amount present in spent fish was "extremely small." Finally, during the present investigation, tests were made upon the liver of the Scottish herring, in Hjort-stage III, which failed to reveal any trace of glycogen or glucose.

## Analytical Results.

### I. *Manx Herring.*

*Samples and Classification.* Particulars of the samples taken for analysis are given in Table I, the reference numbers in the first column being those used in the subsequent analytical tables. Samples were obtained from the

last week in June until the beginning of September, when the local boats engaged in the Manx herring fishery ceased operations. It will be noted that the fish sampled were not all caught on the same ground, those of June, July and August being from the inshore or "low" grounds, while the September catches were made in deeper, offshore, waters. Complete dependence upon available commercial supplies, however, put quite out of the question any selection of a fixed fishing ground. There is some evidence, also [Birtwistle and Lewis, 1922], that the Manx herring shoals of June and July comprise a mixture of sub-types different from that occurring later in the season, but it is not thought that either of these factors is likely to have had any serious influence upon the purely chemical data.

The mean length of the fish, in each sample, is given, as the data may be of use in comparing the present analyses with those of other workers—they are not otherwise made use of.

The age, in years, is simply the number of completed winter-rings on the scales, plus one (*vide* Note, p. 484).

The state of the gonads is given on Hjort's notation:

I. Virgin—fish which have never spawned.
II. Maturing virgins or recovering spents, first stage.
III. Gonads occupying about one-half of the body-cavity.
IV. Gonads occupying about two-thirds of the body-cavity.
V. Full—gonads filling the body-cavity.
VI. Spawning—roe or milt running.
VII. Spent—gonads collapsed.

For the actual purpose of the analyses, however, a broader classification was found to be desirable, especially since many of the samples contained individuals in two successive Hjort stages—four "gonad-groups" have therefore been recognised:

| Gonad-group | Description | Corresponding Hjort stages |
|---|---|---|
| A | Virgin | I |
| B | Filling | II, III |
| C | Full | IV, V |
| D | Spawning and recently spent | VI, VII |

(No samples were obtained representative of Group A.)

*Analytical Tables.* The analyses of the samples, as carried out in order of date, are set out in Table II. For convenience of reference, the results have been calculated as percentages of both the original weight of fresh muscle-substance, and of the dried material. The only points that call for notice at this stage are, first, the remarkable constancy, under all conditions of age and development, of the sum of fat and water, which lies very close to 80 % of the total fresh weight, and, second, the values in the protein columns, which were obtained by multiplying the total nitrogen, found by Kjeldahl's method, by the usual factor, × 6·25. Probably almost wholly as a result of this procedure, the totals in every case fall short of 100 %, the mean defect being

1·75 %. If the factor × 6·9 be used, as indicated by the mean of the values in the column "N" (*vide* p. 474), the totals will be found to lie very close to the theoretical, in every case. Until the precise amount and nature of the non-protein substances present, however, be ascertained, it will be safer to adhere to the accepted factor. The results, of course, retain their relative significance, whatever factor be used, and none of the other components is determined by difference.

Table I. *Manx herrings—Particulars of samples taken for analysis.*

| Ref. No. | Date caught (1923) | Where caught | No. of fish in sample | Mean length (cm.) | Age (years) | State of gonads | Gonad-group |
|---|---|---|---|---|---|---|---|
| 1 | 27 June | "Inshore" | 4 | 24·0 | 4 | II | B |
| 2 | „ | „ | 4 | 22·6 | 3 | II | B |
| 3 | 29 June | „ | 6 | 23·5 | 4 | II | B |
| 4 | 10 July | „ | 4 | 25·1 | 4 | IV, V | C |
| 5 | „ | „ | 4 | 25·6 | 5 | IV, V | C |
| 6 | 13 July | „ (Port Erin Bay) | 5 | — | 4 | IV | C |
| 7 | „ | "Inshore" | 6 | 24·8 | 4 | IV, V | C |
| 8 | 19 July | „ | 10 | 22·6 | 3 | II | B |
| 9 | „ | „ | 10 | 24·1 | 4 | IV | C |
| 10 | „ | „ | 10 | 25·6 | 5 | IV, V | C |
| 11 | 24 July | „ | 15 | 23·3 | 3 | I, II | B |
| 12 | „ | „ | 11 | 23·9 | 4 | III, IV | C |
| 13 | „ | „ | 3 | 25·1 | 5 | II | B |
| 14 | 14 Aug. | „ | 11 | 22·6 | 3 | II, III | B |
| 15 | „ | „ | 11 | 23·9 | 4 | IV, V | C |
| 16 | 28 Aug. | „ | 10 | 22·9 | 3 | III, IV | C |
| 17 | „ | „ | 11 | 24·1 | 4 | V | C |
| 18 | 6 Sept. | 12 m. W. by S. of "Chickens" | 10 | 23·7 | 3 | V | C |
| 19 | „ | „ | 11 | 24·9 | 4 | VII | D |

(NOTE. Inshore or "low" herrings were taken within six miles of land, between Peel and the Calf of Man.)

Table II. *Manx herrings—Analyses of fresh and dry muscle-substance.*

| Ref. No. (Table I) | Percentage composition of fresh muscle-substance | | | | | | Percentage composition of dry muscle-substance | | | "N" (*vide* p. 474) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Water | Fat | Water + fat | Protein (N × 6·25) | Ash | Total | Fat | Protein (N × 6·25) | Ash | |
| 1 | 63·5 | 16·1 | 79·6 | — | 1·49 | — | 44·1 | — | 4·08 | — |
| 2 | 69·2 | 7·5 | 76·7 | 19·7 | 1·60 | 98·0 | 24·4 | 64·0 | 5·20 | 14·5 |
| 3 | 65·5 | 12·4 | 77·9 | — | 1·74 | — | 35·9 | — | 5·04 | — |
| 4 | 65·0 | 15·6 | 80·6 | 16·6 | 1·19 | 98·4 | 44·5 | 47·4 | 3·40 | 14·6 |
| 5 | 62·8 | 18·1 | 80·9 | 16·5 | 1·06 | 98·5 | 48·6 | 44·4 | 2·85 | 14·6 |
| 6 | 65·7 | 13·8 | 79·5 | 17·6 | 1·20 | 98·3 | 40·2 | 51·3 | 3·50 | 14·6 |
| 7 | 67·0 | 11·9 | 78·9 | 18·3 | 1·08 | 98·3 | 36·0 | 55·5 | 3·28 | 14·6 |
| 8 | 67·0 | 12·4 | 79·4 | 17·8 | 1·26 | 98·5 | 37·6 | 54·0 | 3·82 | 14·7 |
| 9 | 64·0 | 16·3 | 80·3 | 16·7 | 1·31 | 98·3 | 45·3 | 46·4 | 3·64 | 14·6 |
| 10 | 61·8 | 19·4 | 81·2 | 15·4 | 1·15 | 97·8 | 50·8 | 40·3 | 3·01 | 14·0 |
| 11 | 65·2 | 14·8 | 80·0 | 16·9 | 1·37 | 98·3 | 42·5 | 48·5 | 3·94 | 14·5 |
| 12 | 65·1 | 15·1 | 80·2 | 16·7 | 1·24 | 98·1 | 43·3 | 47·8 | 3·55 | 14·5 |
| 13 | 65·3 | 14·4 | 80·7 | 16·0 | 1·18 | 97·9 | 41·5 | 46·1 | 3·40 | 14·2 |
| 14 | 67·7 | 10·6 | 78·3 | 18·5 | 1·51 | 98·3 | 32·8 | 57·2 | 4·67 | 14·6 |
| 15 | 65·7 | 13·6 | 79·3 | 17·6 | 1·20 | 98·1 | 39·6 | 51·3 | 3·50 | 14·4 |
| 16 | 65·2 | 13·6 | 78·8 | 18·4 | 1·43 | 98·6 | 39·1 | 52·8 | 4·11 | 14·9 |
| 17 | 63·8 | 16·2 | 80·0 | 16·8 | 1·11 | 97·9 | 44·7 | 46·4 | 3·07 | 14·2 |
| 18 | 65·5 | 14·7 | 80·2 | 17·3 | 1·35 | 98·9 | 42·6 | 50·1 | 3·92 | 15·0 |
| 19 | 63·5 | 17·5 | 81·0 | 15·6 | 1·44 | 98·0 | 48·0 | 42·7 | 3·94 | 14·2 |

*Grouping of Analyses.* On proceeding to group the analytical results according to the age and condition of the samples analysed, certain relations at once become evident—for example, that obtaining, in a general way, between age and fat-content. If the entire series be divided into three groups, containing respectively the 3-year, 4-year, and 5-year old fish, regardless of date, sex, and genital maturity, it will be seen that the older fish have the higher fat-content in the muscle-substance.

| Age of fish (years) | Fat in fresh muscle % | Number of fish upon which estimate is based |
|---|---|---|
| 3 | 12·3 | 60 |
| 4 | 14·9 | 79 |
| 5 | 17·3 | 17 |

A similar but inverse relation can be shown to exist when the water-content is considered. These results are borne out, and rendered even more striking when the data in Table II are fully analysed, so as to show the tissue-composition of the various age-groups, at corresponding levels of sexual maturity. This is done in Table III, where the mean percentages of water, fat and protein at each stage are shown.

Table III. *Manx herrings—Mean percentage composition of fresh muscle, in successive age- and gonad-groups.*

| Age of fish (years) | Gonad-group (*vide* p. 476) | Ref. Nos. (Tables I and II) | Mean % composition | | | |
|---|---|---|---|---|---|---|
| | | | Water | Fat | Protein | Water/fat |
| 3 | B | 2, 8, 11, 14 | 67·3 | 11·3 | 18·2 | 5·96 |
| | C | 16, 18 | 65·3 | 14·2 | 17·9 | 4·60 |
| 4 | B | 1, 3 | 64·5 | 14·3 | — | 4·51 |
| | C | 4, 6, 7, 9, 12, 15, 17 | 65·2 | 14·6 | 17·2 | 4·46 |
| | D | 19 | 63·5 | 17·5 | 15·6 | 3·63 |
| 5 | B | 13 | 65·3 | 14·4 | 16·0 | 4·54 |
| | C | 5, 10 | 62·3 | 18·8 | 15·9 | 3·32 |

Since the proportions of water and fat vary in a complementary manner, the ratio of these values affords the best datum of comparison between the various stages. The mean ratio—water/fat—therefore, is given in the last column of Table III.

These ratios may thus be summarised:

| Age of fish (years) | Ratio: $\frac{\%\ \text{water}}{\%\ \text{fat}}$, in successive gonad-groups | | | |
|---|---|---|---|---|
| | A | B | C | D |
| 3 | — | 5·96 | 4·60 | — |
| 4 | — | 4·51 | 4·46 | 3·63 |
| 5 | — | 4·54* | 3·32 | — |

* This slightly aberrant value is based upon a very small sample only, Ref. No. 13.

From this it is clear that, at any given gonad-stage, the relative amounts of water and fat present in the muscular tissue vary with the actual age of the fish, and further, that the older fish, at any rate within the age- and gonad-range covered by the samples examined, are richer in fat than the younger.

This is a fact of considerable importance, not only economically—as bearing upon the dietetic value of the herring, and its suitability for salting, canning, or other conservation-process, at the various stages in its life-history—but also from a bionomic standpoint. The older fish, with their greater fat-reserve, are able more successfully to withstand a temporary period of adverse conditions, to disperse more widely in search of suitable feeding and spawning grounds, and finally, when other conditions permit, to mobilise more readily their tissue-reserves, whether localised in muscle or liver, for the building-up of the genital products, with consequent earlier maturation. It is believed that these suggestions are in consonance with the observations of biologists on the movements and habits of the herring.

It is also evident from Table III that, in any given age-group, the more sexually mature individuals have the higher fat percentage. This relation, of course, has already been shown to exist, in the case of Manx, Scottish, and Welsh herrings, by Milroy and by Johnstone. The results now obtained, however, appear to indicate, in the case of the 4-year old fish, that the water/fat ratio continues to fall, even after spawning, whereas the above-named workers agree in the view that the maximum fat-content occurs some little time before the actual spawning period. As a matter of fact, the present results are not subversive of the accepted view, for had samples of herring been available in the actual process of spawning, or in the stage immediately preceding it, it is certain that a maximum fat-content, and a minimum water/fat ratio, would have been found to lie between the values in question, in columns C and D. As it is, the value 3·63 is dependent upon one sample only (Ref. No. 19), consisting of eleven fish in a spent condition. As Milroy [1907] has suggested, it is possible, though not likely, that these fish had commenced to feed and fatten, after being so largely depleted during the final period of maturation, when the discharge of the ova or sperms, together with enhanced muscular activity, and complete abstention from food, make an unprecedented demand upon the tissue-reserves.

It is important, at this juncture, to emphasise the fact that the variations undergone by the tissue-fat depend upon the seasonal as well as upon the sexual cycle [Johnstone, 1915]. Thus, the great rise in fat content marking the early stages of maturation in the autumn-spawning Manx herring is a reflection of the increased metabolism consequent upon the rising sea-temperature. The winter-spawning herrings of the Clyde area, on the other hand, mature in cooling water, and their maximum fat-content is considerably lower than that of Irish Sea fish. The generally low fat-values recorded in this paper for Manx herring of 1923, however, are largely due to the deficiency of sea-temperature and sunshine during the summer months of that year, as well as to the large proportion of younger "inshore" fish which have made up the bulk of the season's catch.

Leaving now the consideration of the water/fat ratio, a further interesting relation becomes evident when the variations in the protein-content are

examined. The data in Table III may conveniently be summarised as before:

| Age of fish (years) | Percentage of protein (N × 6·25) in successive gonad-groups | | | |
|---|---|---|---|---|
| | A | B | C | D |
| 3 | — | 18·2 | 17·9 | — |
| 4 | — | — | 17·2 | 15·6 |
| 5 | — | 16·0 | 15·9 | — |

From this it is clear that the percentage of protein in the muscle substance falls off with advancing age, and with progressive maturation of the gonads, though the range of variation is much less than that encountered in the case of fat and water. It is not easy to account for these variations. They may, in part, be due to the presence of some substance, varying in nature or amount from time to time, in which the nitrogen-content differs progressively from the 16 % assumed to be present in the "standard" protein; though the fairly constant values of "N" (Table II) calculated for the various stages do not lend support to this view. They may, on the other hand, in so far as they represent actual fact, indicate a conversion from protein to fat, as one stage in the tissue-changes accompanying the development and maturation of the genital products.

The diminution of protein-content in the successive age-groups, concurrently with rising fat-content, may be an expression of the same dynamic equilibrium between these substances.

Finally, the proportion of non-volatile inorganic matter in the various samples has been studied, but no definite relation appears to exist between this value and the age and gonad factors so far considered.

## II. *Scottish (Clyde Area) Herring.*

As in the case of the Manx herring, a list (Table IV) is subjoined, giving the date, station and condition of each sample examined.

Table IV. *Clyde herrings—Particulars of samples taken for analysis.*

| Ref. No. | Date caught (1922) | Where caught | No. in sample | Mean length cm. | Mean girth cm. | Mean weight g. | Sex | Hjort stage |
|---|---|---|---|---|---|---|---|---|
| 1 *a* | 5 Sept. | Skipness | 9 | 25·6 | 12·8 | 140·9 | F. | II |
| 1 *b* | ,, | ,, | 4 | 25·9 | 13·0 | 139·8 | M. | II |
| 2 *a* | 21 Sept. | — | 9 | 25·5 | 12·8 | 138·5 | F. | III |
| 2 *b* | ,, | — | 6 | 25·9 | 12·6 | 139·0 | M. | III |
| 3 *a* | 4 Oct. | Imachar Pt. | 12 | 27·0 | 13·9 | 162·0 | F. | III |
| 3 *b* | ,, | ,, | 11 | 28·0 | 14·3 | 184·0 | M. | IV |
| 4 *a* | 18 Oct. | — | 10 | 27·4 | 13·7 | 172·6 | F. | III |
| 4 *b* | ,, | — | 15 | 27·2 | 13·6 | 175·0 | M. | IV |

Although the ages of the fish in the actual samples were not determined, it seems quite clear, from collateral scale-readings made upon fish from the same shoals, that 3-, 4-, and 5-year old individuals were included in the samples. The analyses, as carried out by Mr J. Secker, in order of date, are set out in Table V, from which it will be noted that the sexes have been

separately examined, and that in each case three different tissues—muscle, liver, and gonad—have been selected for analysis.

It will be convenient, at the outset, to consider only the water-, fat-, and protein-content of these tissues, deferring until a later stage the phosphorus-values and their significance.

Table V. *Clyde herrings—Analyses of fresh tissues.*

| | | | | Protein (N × 6·25) | | | Phosphorus (as $P_2O_5$) | | |
|---|---|---|---|---|---|---|---|---|---|
| Ref. No. | Tissue | Water* % | Fat* % | Using* dry fat-free residue % | Using fresh tissue % | Coag. protein % | Using* dry fat-free residue % | Using fresh tissue % | Total* % |
| 1 *a* | Muscle | 64·5 | 16·9 | 17·5 | — | 14·1 | 0·65 | — | 99·6 |
| | Gonad | 70·5 | 3·6 | 25·1 | — | — | 0·50 | 0·81 | 99·7 |
| | Liver | 63·4 | 15·9 | — | — | — | — | 0·73 | — |
| 1 *b* | Muscle | 63·5 | 18·7 | 17·2 | — | 13·0 | 0·45 | 0·59 | 99·9 |
| | Gonad | 76·8 | 3·7 | 18·6 | — | — | — | 0·82 | (99·1) |
| | Liver | 63·2 | 23·1 | — | — | — | — | 0·58 | — |
| 2 *a* | Muscle | 63·4 | 18·6 | 17·0 | — | 13·5 | 0·42 | 0·56 | 99·4 |
| | Gonad | 67·0 | 4·0 | 25·1 | — | — | 0·71 | 0·86 | 96·8 |
| | Liver | 66·8 | 13·4 | — | — | — | 0·54 | 0·76 | — |
| 2 *b* | Muscle | 62·0 | 20·5 | 16·6 | — | — | 0·46 | 0·57 | 99·6 |
| | Gonad | 76·6 | 3·8 | 20·1 | — | — | 1·12 | 1·30 | 101·6 |
| | Liver | 61·2 | 21·2 | — | — | — | 0·43 | 0·66 | — |
| 3 *a* | Muscle | 63·2 | 18·7 | 17·3 | 19·6 | 14·1 | 0·47 | 0·57 | 99·7 |
| | Gonad | 66·8 | 2·5 | 25·8 | 27·4 | — | 0·65 | 0·85 | 95·8 |
| | Liver | 70·5 | 9·5 | — | — | — | 0·61 | 0·85 | — |
| 3 *b* | Muscle | 62·7 | 19·4 | 17·3 | 17·4 | 13·5 | 0·38 | 0·57 | 99·8 |
| | Gonad | 75·3 | 3·0 | 22·4 | 23·2 | — | 1·02 | 1·17 | 101·7 |
| | Liver | 67·2 | 15·3 | — | — | — | 0·40 | 0·59 | — |
| 4 *a* | Muscle | 63·0 | 18·7 | (15·3) | 18·6 | 15·3 | 0·35 | 0·56 | (97·4) |
| | Gonad | 66·6 | 2·2 | 26·3 | 27·6 | — | 0·56 | 0·86 | 95·7 |
| | Liver | 71·4 | 8·9 | — | — | — | 0·51 | 0·76 | — |
| 4 *b* | Muscle | 60·3 | 22·4 | 16·8 | 17·5 | 13·3 | 0·39 | 0·55 | 99·9 |
| | Gonad | 75·3 | 3·4 | 23·1 | 24·4 | — | — | 1·17 | (101·8) |
| | Liver | 68·9 | — | — | — | — | — | 0·59 | — |

* Totals are those of values in columns marked with an asterisk.
Values enclosed in brackets are omitted from calculation of averages.

Table VI. *Clyde herrings—Mean percentage composition of fresh muscle.*

| | Hjort stage (p. 476) | Ref. Nos. (Table V) | Mean percentage composition: Water | Fat | Water + fat | Protein* (N × 6·25) | Water/fat |
|---|---|---|---|---|---|---|---|
| *Females:* | | | | | | | |
| | II | 1 *a* | 64·5 | 16·9 | 81·4 | 17·5 | 3·82 |
| | III | 2 *a*, 3 *a*, 4 *a* | 63·2 | 18·7 | 81·9 | 17·2 | 3·39 |
| *Males:* | | | | | | | |
| | II | 1 *b* | 63·5 | 18·7 | 82·2 | 17·2 | 3·40 |
| | III | 2 *b* | 62·0 | 20·5 | 82·5 | 16·6 | 3·02 |
| | IV | 3 *b*, 4 *b* | 61·5 | 20·9 | 82·4 | 17·1 | 2·97 |

* The protein values are those determined upon the dry, fat-free residue.

*Composition of Muscular Tissue.* The range of gonad-development covered by the samples is too small to admit of their being arranged in "gonad-groups," as in Table III, but if the analyses of the muscular tissue be placed in order

of the successive Hjort stages, as shown in Table VI, the same general relation between the water/fat ratio and sexual maturity becomes evident, as has already been shown to exist in the case of Irish Sea herring.

It will be noted, moreover, that the relation is independent of sex, the slight disparities between the analyses of male and female tissue lying within the bounds of error inherent in sampling and manipulation. The fact that the water/fat ratios are considerably lower than those observed in the Manx samples (Table III) is doubtless to be associated with differences of race and environment (*vide* p. 479); to this cause may be traced the fact that the sum of the water and fat percentages averages 82·1, in contrast with the 80·0 % "constant," noted in the case of the Manx samples. The variations in the protein values, also, show the same downward trend with advancing development of the gonads.

*Composition of Gonads.* In Table VII the composition of the ovaries and testes is given, as determined over the same range of development.

Table VII. *Clyde herrings—Mean percentage composition of fresh gonads.*

| | Hjort stage (p. 476) | Ref. Nos. (Table V) | Mean percentage composition | | |
|---|---|---|---|---|---|
| | | | Water | Fat | Protein* (N × 6·25) |
| *Females:* | II | 1 *a* | 70·5 | 3·6 | 25·1 |
| | III | 2 *a*, 3 *a*, 4 *a* | 66·8 | 2·9 | 25·7 |
| *Males.* | II | 1 *b* | 76·8 | 3·7 | 18·6 |
| | III | 2 *b* | 76·6 | 3·8 | 20·1 |
| | IV | 3 *b*, 4 *b* | 75·3 | 3·2 | 22·8 |

* The protein values are those determined upon the dry, fat-free residues.

The results are too scanty to enable any definite conclusions to be drawn, but so far as they go, they indicate that the male organs are richer than the female in both water and fat, and correspondingly poorer in protein; it is also evident that a diminution of both water and fat content, and a rise in protein content, is associated with the early phases of maturation in both sexes. No constancy is to be observed in the combined percentages of water and fat.

A comparison of the analyses of muscle-substance (Table VI) with those of the gonads (Table VII) affords no significant evidence which would justify the view that transference of fat or protein takes place from the one tissue to the other. Apart, however, from fatty reserves present in the muscle-substance, a considerable amount of solid or semi-solid fat may be observed, in maturing herrings, investing the intestine and other organs. This—the so-called "mesenterial fat"—may be of great value at a time when the available or ingested food-supply fails to meet the energy- and tissue-requirements of the rapidly maturing gonads. The fat, however, is not in a form capable of immediate translocation, and when rendered available by enzyme-activity, it should be possible to detect its even transitory presence in muscle or liver.

*Composition of Liver.* From Table VIII it will be seen that the liver is rich in fat, more so in the male than in the female, and that there is a marked

falling-off in fat-content concurrently with the maturation of the genital products. It is not improbable, therefore, that the metabolic demands of the growing gonads are met, in the first instance, by the fatty reserves of the liver, while at a later stage, the stores of mesenterial fat may be called upon. There is no evidence that the fat of the muscular tissue undergoes depletion, until shortly before the time of spawning.

Protein-values are not available, since no nitrogen determinations were made upon the liver.

Table VIII. *Clyde herrings—Mean percentage composition of fresh liver.*

| | Hjort stage (p. 476) | Ref. Nos. (Table V) | Mean percentage composition: Water | Fat |
|---|---|---|---|---|
| *Females:* | II | 1 *a* | 63·4 | 15·9 |
| | III | 2 *a*, 3 *a*, 4 *a* | 69·6 | 10·6 |
| *Males:* | II | 1 *b* | 63·2 | 23·1 |
| | III | 2 *b* | 61·2 | 21·2 |
| | IV | 3 *b*, 4 *b* | 68·1 | 15·3 |

*The Rôle of Phosphorus.* The determinations of phosphorus in muscle, gonad, and liver, are few and erratic. Further experimental work, improved technique, and a much wider range of samples are needed before any clear idea can be obtained of the rôle of this element in the herring's metabolism. The results in Table IX, so far as they go, appear to indicate that the developing gonads make a heavy demand for phosphorus, the testes having relatively the higher requirement. This is in agreement with the findings of Milroy [1908].

Table IX. *Distribution of phosphorus in the tissues of the herring.*

| | | Muscle | | Gonad | | | Liver | | |
|---|---|---|---|---|---|---|---|---|---|
| Hjort stage | Ref. Nos. | % $P_2O_5$ using fresh tissue | % $P_2O_5$ using dry fat-free residue | % $P_2O_5$ using fresh tissue | % $P_2O_5$ using dry fat-free residue | Ratio*: $\frac{\text{Gonad } P_2O_5}{\text{Muscle } P_2O_5}$ | % $P_2O_5$ using fresh tissue | % $P_2O_5$ using dry fat-free residue | Ratio*: $\frac{\text{Gonad } P_2O_5}{\text{Liver } P_2O_5}$ |
| *Females:* | | | | | | | | | |
| II | 1 *a* | — | 0·65 | 0·81 | 0·50 | 0·92 | 0·73 | — | — |
| III | 2 *a*, 3 *a*, 4 *a* | 0·56 | 0·41 | 0·86 | 0·64 | 1·53 | 0·79 | 0·56 | 1·09 |
| *Males:* | | | | | | | | | |
| II | 1 *b* | 0·59 | 0·45 | — | — | — | 0·58 | — | — |
| III | 2 *b* | 0·57 | 0·46 | 1·30 | 1·12 | 2·28 | 0·66 | 0·43 | 1·97 |
| IV | 3 *b*, 4 *b* | 0·56 | 0·39 | 1·17 | — | 2·09 | 0·59 | — | 1·98 |

N.B. The percentages are in every case calculated on the fresh (wet) weight of the tissues.

* Based upon the values obtained, using fresh tissue.

A wide disparity is to be observed between the $P_2O_5$ determinations made respectively upon the fresh and extracted tissues. If the extent of the disparity is regarded as in any way indicative of the amount of phosphorus present as phosphatide, or other fat-soluble compound (p. 475), the suggestion may be hazarded that these lecithin-like bodies play an important part in the phosphorus-metabolism of the herring, and in the up-building of the nucleoproteins of the mature genital products. It is not without significance that the phosphatide-content of the liver, as judged on the above basis, appears to be higher than that of the other tissues.

## Note.

*Estimation of the Age of Herrings.* Scales are taken from each fish, cleaned, and mounted dry on a glass slide. The number of opaque or semi-opaque rings is counted, care being taken to distinguish between the fine concentric markings due to the lamellar structure of the scale, and the real "winter-rings" denoting the years of growth.

No difficulty is usually experienced when the number of rings is less than four or five, but when more numerous, the rings are crowded near the edge of the scale, and considerable experience is needed before accurate counts can be made. When a doubtful scale occurs, it is stained in silver nitrate and examined in polarised light—this materially assists in the elimination of false rings.

It should be remembered that autumn and winter spawned herrings exist for about the first six months of their life without scales, so that the first area on a scale is laid down during the period May to September, which is considered to be the scale-growing period. The naked period is not represented on the scale, and should not be overlooked when estimating the actual age of the fish. A winter-ring only becomes definitely a ring when it is bounded by a summer growth of an appreciable extent, so that by the time this ring (in a one-ring fish) is surrounded by a summer growth the fish is well on the way towards completing two years of life; similarly with older scales the actual age in years is taken as being one more than the number of winter rings on the scale [Birtwistle, 1921; Birtwistle and Lewis, 1923].

## Summary.

Analyses have been made upon a series of herring samples, taken throughout the Manx fishing season of 1923, with a view to ascertaining the relation, if any, between age and the chemical composition of the tissues.

The "winter-rings" upon the scales have been taken as a criterion of age, and the samples examined have been homogeneous as regards age and sexual maturity.

At any given stage of sexual maturity, the water-, fat- and protein-content of the fresh muscle-substance are dependent upon age, the older fish containing a lower percentage of water and protein, and a higher percentage of fat.

The variations in composition, with age, resemble closely those associated with the successive stages of sexual maturation, established by Milroy and Johnstone.

Analyses made upon a limited series of herring-samples from the Clyde area, in 1922, indicate the general distribution of water, fat, and protein, between muscle, liver, and gonad, during the middle or "filling" period of maturation. They suggest that the metabolic demands of the growing gonads are met, in the first instance, by the fatty reserves of the liver; the muscular tissue is not depleted until shortly before spawning occurs.

Determinations of phosphorus were made in the case of the Scottish samples. Further work and improved technique will be needed before definite conclusions can be drawn as to the part played by this element, but it is clear that the developing gonads have a high phosphorus-requirement, and that the phosphatides (lecithin, etc.) figure largely in the tissue-changes and translocations associated with maturation.

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