XII. THE RÔLE OF GLYCOGEN, LECITHIDES, AND FATS IN THE REPRODUCTIVE ORGANS OF ECHINODERMS.

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In the course of a research upon the protamines and histones of the spermatozoa of *Echinus esculentus* in which the reproductive organs (gonads) were extracted with hot alcohol, we were struck by the enormous amount of fatty matter removed by the hot alcohol. This separated out in drops and formed a thick layer at the bottom as the alcohol cooled. This appeared to us an unusual result to obtain from a male gland possessing only a reproductive function. It was still more surprising to find the aqueous extract made later on, in the process of extraction of the protamines, heavily laden with glycogen as shown by a marked pearly translucence, and confirmed by a marked iodine reaction even after manifold dilution. In both of these respects, the extracts of the gonads might well have been those from a liver or hepato-pancreas in a vertebrate or higher invertebrate, so rich were they in the usual products of metabolism. In the echinodermata, the gonads form by far the preponderating part of the soft tissues of the animal, and contain at least three-fourths of the organic matter of the organism. Also there exists in these animals no structure resembling a liver, or other metabolic gland.

It was accordingly determined to make analyses of the fatty constituents and glycogen of the gonads to elucidate their functions in this respect. For this purpose experiments were instituted to observe the effects of seasonal variation in April and in August—the period of sexual activity being in April; to keep the animals in batches for some weeks in the fed and unfed conditions respectively; and to test whether the gonads contained any diastatic enzymes.

The exceedingly slow metabolism of these organisms during even a lengthy period in which they were unfed, precluded us however from observing whether the reserve stores of fat and glycogen of the gonads could

be utilized for the purposes of the general metabolism of the animal as a whole. As is shown in another research [Moore, Edie, Whitley and Dakin, 1912, p. 287] upon the metabolism of marine organisms, the amount of organic matter required in twenty-four hours by an average-sized echinus is only 4.2 milligrams, and as the gonads contain about five grams of dry organic matter it is impossible to say whether these are acting as reservoirs for the minimal demands of the organism during deprivation of food even for a month or more. For example, 4.2 milligrams of organic matter oxidized daily, is equivalent to 30 milligrams of moist weight, and for one month, or 30 days, this would amount to a loss in weight of only 0.9 g. This much, however, is certain, that the gonads are very retentive of their stored-up food supplies, such as fats and glycogen, for after even three weeks' complete deprivation of food, the gonads are still very rich in both oils and glycogen, and even quantitative determinations show no constant difference between the fed and the unfed organisms. The unfed specimens are alive and well at the end of the period and commence at once to feed greedily when offered suitable food such as ordinary laminaria.

It is not to be supposed that the metabolism of the echinus is so excessively slow under normal conditions; this is only a dormant condition which can be assumed when there is necessity for it. When the organisms are first placed in captivity without food, there is an enormous output of faeces for the first two days, afterwards the water in the tank is practically free for the rest of the period. The animals also develop an appetite, which was shown by an attack upon the wooden bottoms of the tanks in which the unfed echini were stored. The unfed animals supported themselves in the stream of running water by the tube-feet just like the fed ones and appeared quite active and well in every respect.

The echini fed in the laboratory upon laminaria and other sea-weeds, ate quite vigorously and produced a large output of faeces daily, but at the end of the period, the extracts from the two sets of gonads, of fed and unfed, were very much alike in storage content. There may have been, however, an increase in bulk of the gonads, of those which were fed, for the size of the gonads varies considerably and they cannot be weighed at the beginning and end of the experiment.

Another important point to remember is that there is no trace whatever to be detected of a diastatic enzyme.

A gonad rich in glycogen may be left for some hours either at air temperature, or heated in a bath to 35°, for the same time, and at the end not a trace of sugar is obtainable.

It may even be left for a period of two days with the same result. But, extraction at the end shows the glycogen to be present in large amount, and on boiling with dilute acid a copious reduction with Fehling's solution is at once obtained.

The diminished metabolism during fasting, compared with the rapid uptake of food while fed, the persistence of fat and glycogen after long starvation, and the absence of a diastatic enzyme, when all considered together, and in conjunction with the relatively huge size of the reproductive organs, indicate that the stored fat and glycogen are intended for the internal metabolism of the gonads themselves and to subserve the enormously developed reproductive functions.

The main bulk of the metabolized food would also appear to be devoted to the nourishment and upbuilding of the reproductive organs. These organs appear during the resting season to build up rich nutritive material and then discharge this during the active season, when a diastatic ferment may perhaps be temporarily developed.

It is remarkable that a few months after the active season, as in August (the period of activity having been the end of March and earlier part of April), the gonads are as large and full as before activity, and richly charged with oils and glycogen. While being fed the uptake of material must be many times the amount required for locomotion and other physiological purposes in the unfed condition, as shown by the amount of oxygen used daily by the unfed animal. If it may be assumed that the unfed animal requires about as much energy for these purposes as the fed animal, as would seem to be shown by the activity of the two being similar, then it may be concluded that at least nine-tenths of the energy of the food taken up by the echinus is conserved for reproductive purposes.

In regard to the carbohydrate and fatty metabolism, there is no noticeable difference between the male and female gonads, both are rich in oils and in glycogen.

A considerable amount of lecithides was in all cases found admixed with the fats. The iodine value for both lecithides and oils was high, showing a marked degree of lack of saturation. In this respect the fats of the echinus resemble the fish-oils, or fish liver-oils.

Thus, for the oils, iodine values varying from 130 to 190 were obtained, and for the lecithides, values of 70 to 120. These high iodine values suggest that these fatty constituents may possess therapeutic values similar to those of cod-liver oil in wasting diseases.

METHODS OF EXTRACTION AND ANALYSIS.

In the earlier experiments carried out at Easter 1911, only the fats, oils and lecithides as extracted by thorough treatment (*a*) with cold and (*b*) with hot alcohol afterwards, were obtained and analysed.

The work was resumed in August and September 1911, and Easter 1912, and then two methods chiefly were employed. In the first method the glycogen only was estimated, by boiling out the gonads twice with water acidulated with acetic acid, after which a third decoction showed no opalescence and gave very little reaction with iodine. The two extracts were added together, precipitated by the double volume of alcohol, washed with 70 per cent. alcohol by decantation, and preserved in this for some months, after which the precipitated impure glycogen was digested with strong caustic potash, as in Pflüger's method described in the concluding part of the second method below, followed by precipitation with alcohol, washing with alcohol, drying and weighing. In several cases, the amount of glycogen present was finally determined by hydrolysis to glucose, reduction by excess of Fehling's solution, and gravimetric determination of the CuO. When this was done it was found that the product of the Pflüger's method was never pure glycogen, but varied from 63 to 93 per cent. pure.

Both results are given alongside each other in the tables below for comparison. The nature of the impurity was not determined, but it indicates that this method of determining glycogen should be used with caution, and always followed by a hydrolysis and sugar determination.

Second Method. In this, the final method used, which was found by far the most convenient, oils, lecithides, and glycogen were all determined in the same specimen. At the end of the preliminary treatment of the organisms at the Biological Station, the shells were broken open by chipping round in a sectional plane, midway between mouth and anus, and the sex determined by examining a smear from a gonad with a low power of the microscope.

The echini were thus sorted into two heaps, the five gonads were removed from each echinus, and two heaps made of male and female gonads respectively. The weight of each quantity in the fresh condition was taken, and they were then broken up and placed in about three times their volume of absolute alcohol. In this condition they were transported to Liverpool and after some months were then extracted for oils and lecithides, and for glycogen respectively by the following method.

The alcohol was decanted and filtered off, without previous heating. A

fresh quantity of absolute alcohol was then added to the tissue which was previously broken up as finely as possible, and mechanical agitation in a bottle on a shaker was used for some hours. This second quantity of alcohol was filtered off as before, and when mixed with the first, the mixture formed the "cold alcoholic extract."

The "hot alcoholic extract" was obtained similarly by two extractions with boiling alcohol, decantation and filtration.

Each alcoholic extract was subsequently treated in precisely the same fashion for determination (a) of salts and extractives, (b) lecithides, and (c) fats and oils, as follows:

The alcoholic extract was evaporated till all alcohol had been removed, and the total weight was taken. The residue was exhausted by repeated portions of methylated ether, which portions were all united. The residue from the ether consisted of salts, creatine, etc., and this was weighed separately. The ethereal extract was evaporated down cautiously to a thin syrupy consistency, acetone in excess was then added to precipitate the "lecithide" fraction, this was dried and weighed and constants determined. The portion soluble in acetone gave the product described under the heading oils and fats. It is, of course, only claimed that this is a rough separation. The labour of a complete separation in so many experiments would have been inordinately great, and the figures for the constants in the two respective columns (1) lecithides, and (2) oils and fats, show that these two fractions represent a fairly complete separation of the lecithides from the oils. The weight, saponification value, and iodine value, were determined in each case.

The dried tissue after removal of fats, etc., as above described, was weighed and then digested for three hours on a steam bath with 40 per cent. solution of caustic potash, which, as discovered by Pflüger, leaves the glycogen intact while it decomposes proteins. After cooling, twice the volume of absolute alcohol was added, so precipitating the glycogen. The glycogen was filtered off through a Gooch crucible provided with a filter of finest woven silk bolting such as is used for flour sifting. This was found to be much preferable to glass-wool, or asbestos, and may be recommended as a filtering medium for alkaline fluids. The precipitate is then washed with a 15 per cent. solution of caustic potash in a 66 per cent. solution of alcohol, as recommended by Pflüger. Next it is washed through into solution in a fresh receiver with hot water. The aqueous solution is again filtered, reduced in volume by evaporation if necessary, and finally precipitated by twice its volume of absolute alcohol, filtered, washed with two-thirds alcohol, dried and weighed. Finally, the content of glycogen in the product is determined

TABLE I.
Determinations of Lecithides and of Fats and Oils in Ripe Ova and Spermatozoa of Echinus esculentus.

Material and extractive	Total weight of extract	Salts, of creatine, etc. extractives	Lecithide values			Fat and Oil values			Dry weight of residue after complete alcoholic extraction
			Total weight	Saponi- fication value	Iodine value	Total weight	Saponi- fication value	Iodine value	
OVA.									
Exp. I. Ripe Ova, 580 g. moist weight:									
<i>Cold alcohol</i> ...	8.56	2.90	2.10	259	73.4	2.85	205	166	grams 27.95
Percentages on moist weight ...	1.48	0.50	0.36	—	—	0.49	—	—	
<i>Hot alcohol</i> ...	11.44	0.35	2.34	207	76.0	8.13	192	136	
Percentage ...	1.97	0.06	0.40	—	—	1.40	—	—	
Exp. II. Ripe Ova, 332 g. moist weight:									
<i>Cold alcohol</i> ...	7.10	3.50	1.10	207	84.0	2.15	188	133	12.05
Percentage ...	2.14	1.06	0.33	—	—	0.65	—	—	
<i>Hot alcohol</i> ...	8.10	1.20	1.70	229	82.0	4.23	195	145	
Percentage ...	2.44	0.36	0.51	—	—	1.30	—	—	
SPERMATOOZOA.									
Exp. III. Ripe Spermatozoa, 700 g. moist:									
<i>Cold alcohol</i> ...	9.04	1.04	2.60	176	86.1	4.61	201	140	21.7
Percentage ...	1.29	0.15	0.37	—	—	0.66	—	—	
<i>Hot alcohol</i> ...	10.62	0.72	0.50	180	77.0	9.08	172	131	
Percentage ...	1.52	0.10	0.07	—	—	1.30	—	—	
Exp. IV. Ripe Spermatozoa, 235 g. moist:									
<i>Cold alcohol</i> ...	3.10	0.48	0.65	186	90.7	1.40	200	166	(Not taken)
Percentage ...	1.31	0.20	0.28	—	—	0.60	—	—	
<i>Hot alcohol</i> ...	7.67	0.75	1.80	190	81.0	3.33	197	153	
Percentage ...	1.28	0.32	0.77	—	—	1.43	—	—	

by hydrolysis, and gravimetric determination by precipitation in excess of Fehling's solution.

Experiments of Summer 1911 (August and September). The first four experiments to be described in this series were in regard to content of glycogen only, and the effects of lack of food upon the glycogen content of the gonads. The gonads in all these experiments are taken at an inactive sexual period. The ova in the females at this time of year are unripe and small, and formed eggs are small in number relatively to other tissue; the spermatozoa in the males are alive and moving, but small.

Thirty echini of medium and large size only were taken from Port Erin Breakwater at low tide on August 22, all were kept in a large tank filled with running sea-water until August 24 (about 48 hours), not being fed in the interval.

Twenty of the echini were then opened and the gonads examined for sex, twelve were found to be females and eight were males. The female and male gonads separately were at once treated for glycogen separation as will presently be described in Expts. V and VI. The remaining ten echini were kept in the tank in fresh running sea-water without any food until Sept. 11, that is for a period of twenty days in all; they were alive and well at the end of that period, and were then opened, when gonads, and all the other organs, presented quite a healthy and well-fed appearance: the intestines were of course empty. On examination of these for sex, four were found to be females and six were males. The two masses of female and male gonads were then at once treated for extraction of glycogen in the fresh condition with the results shown in Expts. VII and VIII.

Experiment V. The gonads from the twelve females described above weighed 440 g. in the moist condition or an average of 36.6 g. per animal. As subsequent determination of dry weight of a given weight of moist gonad gave a solid content of 17.2 per cent., this weight represents 75.7 g. of dry solid. This dry solid has salts present in the proportion of sea-water in invertebrates so that the amount of dry organic matter is about 62.5 g.

The material was mashed up into a soft pulp, and the proteins coagulated by dropping the pulped mass into 400 c.c. of boiling one per cent. acetic acid in distilled water, and boiling for five minutes. The fluid was then filtered off through fine silk gauze and pressed out. The residue of tissue was pounded up and extracted with 500 c.c. of distilled water in two portions. The last filtrate was almost clear of glycogen, and gave only a trace of reaction with iodine. The first filtrate in dilute acetic acid was a strongly opalescent solution but without obvious suspension. This was again filtered through

two folds of fine silk, and a small portion of the filtrate which was still strongly opalescent gave a strong port-wine colour with iodine, and on boiling for five minutes with 3 per cent. hydrochloric acid gave a copious reduction with Fehling's solution.

The main volume of the acetic acid solution and the washings were united and precipitated with twice the volume of absolute alcohol giving an abundant white precipitate settling easily. This was washed, after decantation, with more two-thirds alcohol, and carried under alcohol to Liverpool, where it was later passed through the Pflüger process for purification of the glycogen and analysed. The weight of glycogen obtained was 7.44 g., but hydrolysis and a gravimetric Fehling determination showed this to be only 79 per cent. pure glycogen giving 1.33 per cent. glycogen on the moist weight, or the glycogen in the gonad is 9.36 per cent. of the total organic matter.

Experiment VI. The eight sets of male gonads weighed 258 g., thus averaging 32.25 g. per animal. Of this quantity, 200 g. were taken for glycogen extraction, corresponding to 28.2 g. of dry organic matter. The tissue was put through exactly the same process as used for the female glands, and gave the same appearances and the same qualitative tests. The filtration of the dilute acetic acid solution was more difficult, probably on account of the presence of the minute spermatozoa, but obviously there was much glycogen present, as shown by fine opalescence and iodine test. After filtration through fine silk it was precipitated by two volumes of alcohol as before, and carried off for analysis. Any spermatozoa present in the original acetic acid precipitate would be destroyed by the subsequent boiling with strong alkali in the Pflüger process. The amount of glycogen obtained was 3.02 g., and this was found on hydrolysis to be 81.4 per cent. pure, corresponding to 1.51 per cent. of crude and 1.23 per cent. of pure glycogen in the moist gonad, or to 8.66 per cent. of the total organic matter.

It is thus seen that the percentage of glycogen is much the same in the male and female gonads, corresponding to 9.36 per cent. in the female and 8.66 per cent. in the male of the dry organic matter.

Experiment VII. The four sets of gonads from the females which had been kept unfed in the tank for 20 days weighed 122 g., or an average of 30.5 g. per echinus, while Expt. V shows that the twelve sets of gonads of females killed without previous abstention from food weighed 440 g., an average of 36.6 g. The difference is not great and is probably quite fortuitous, as the content of glycogen indicates no effects of the twenty-days' fast.

The process of extraction was exactly the same as in Expts. V and VI,

and yielded similar qualitative results. The yield of crude glycogen was 2.09 g. but this, for some unknown reason, showed only 62 per cent. of glycogen on hydrolysis, corresponding to 1.06 per cent. of the moist gonad, or 7.47 per cent. of the total organic matter. This is a somewhat smaller figure but the decrease is too small to mean any real demand on the gonads for reserve food by the rest of the organism during the period of inanition, also they are still very rich in glycogen. Moreover, the result is the reverse in the experiment with the males now to be described.

Experiment VIII. The six sets of gonads from males kept without food in the tank of running sea-water for 20 days weighed 237 g., thus averaging 39.5 g. per animal. These were extracted with dilute acetic acid and precipitated with alcohol at Port Erin on similar lines to the previous experiments, and the precipitated crude glycogen was afterwards worked up by Pflüger's method at Liverpool, and controlled by hydrolysis and estimation of glucose. There were obtained 4.45 g. of glycogen, shown by hydrolysis to be 82.8 per cent. pure; this works out at 1.54 per cent. of pure glycogen in the moist gonad, or 10.84 per cent. of the organic matter. It is here seen that the glycogen in the unfed males is somewhat higher than in the fed males of Expt. VI; but as the females are in the reverse direction by about a similar amount, the proper conclusion is that the four experiments demonstrate no appreciable disappearance of glycogen from the resting gonads of male or female as a result of a fast of twenty days. All four experiments concordantly show that the reproductive organs of *Echinus esculentus* are very rich in glycogen.

A second catch of 60 well-grown individuals was made from Port Erin Breakwater on August 25 at low water with which the remaining experiments of this series were carried out. All these were left over-night in a large tank through which there ran an abundant supply of fresh sea-water. Next morning a large amount of faecal matter obviously chiefly of vegetable origin was found on the bottom of the tank. On the morning of the day after capture (Aug. 26), 15 individuals taken indiscriminately were opened, examined for sex, and the gonads assembled in two lots, one male, the other female. Out of these 15 specimens, six were females, eight males, and one was rejected as being in an abnormal condition. The six female sets of gonads weighed 154 g., or 26.6 g. per individual; the eight male sets weighed 235 g., or 29.4 g. per individual. The mass of gonads in each case was sampled out into three portions which were used as follows: (1) was at once placed directly in alcohol, for determination later of oils and fats, lecithides, and glycogen, (2) was at once extracted with dilute acetic acid and

precipitated with alcohol, etc. for glycogen determination, as in the preceding four experiments, and (3) a smaller quantity was dried on a bath for determination of total solid matter in the fresh tissue of the gonad.

Out of the 154 g. of female gonad, 74.5 g. were placed in alcohol (see Expt. IX b), 61.2 g. were extracted fresh with dilute acetic acid (see Expt. IX a), and 14.26 g. were taken for determination of total solids, which were found to amount to 17.2 per cent., as the glands contain approximately 3 per cent. of inorganic salts, on the basis that their fluids are isotonic with sea-water this leaves 14.2 per cent. of dry organic matter in the female gonad.

Similarly the eight male gonads weighing 235 g. were divided into 75.1 g. placed directly in absolute alcohol (see Expt. X b), 134.4 g. extracted fresh with dilute acetic acid (see Expt. X a), and 12.75 g. taken for determination of total solids. The percentage of total solids found was 16.8, and after deduction for inorganic constituents this leaves 13.8 per cent. of dry organic matter in the tissue of the male gonad. This lies very close to the figure for the female organ given above, and the values of 17 per cent. for total solids, and 14 per cent. for organic solids, may be taken as an average.

The remaining 45 individuals of the second catch (Aug. 25) were used, as follows, for experimentation in the laboratory. They were divided into three lots of 15 individuals each, the sizes being arranged as far as possible so as to give no advantage to any one batch¹. The first batch were left unfed in the large tank (see Expts. XI and XII). The second and third batches were removed in order to have plenty of room and fresh running sea-water, to a series of smaller tanks forming part of a fish-hatching apparatus not at the time in use. The thirty echini were distributed over ten of these tanks, three in each tank. Fifteen of these echini were fed on fresh laminaria gathered on the shore (see Expts. XII and XIII) and they ate a surprisingly large amount of it daily. The other fifteen, forming the third batch, received no food of any kind during the period of the experiment (see Expts. XIV and XV). As some index of the rate of feeding of the echini supplied with laminaria there may be mentioned the amount of faeces found in the tanks.

The faecal masses were washed into a graduated 100 c.c. measure, allowed to sediment, and the volume of the sediment measured. The volumes found were as below. At this time in the experiment a few of the echini had perished, as noted below:

Tank No. I. Two echini, 45 c.c. of faecal sediment in three days.

¹ As a matter of fact it frequently happens that heavier gonads are obtained from a medium-sized than from a large echinus, so that this precaution is scarcely required so long as no individuals below a certain size are taken in the first instance.

Tank No. II. Two echini, 35 c.c. of faecal sediment in three days.

Tank No. IV. Three echini, 100 c.c. of faecal sediment in three days.

The amount of laminaria eaten was not actually weighed, but it was quite a surprising amount for organisms of so slow a metabolism, and was evidently chiefly being used for storage in the gonads. The unfed batches of these echini produced no faeces, after the first two days or thereabouts, and at the end of the experiment it was noticed that in the tanks of the fish-hatching apparatus, which were made of planed wood, in each case four or five bitten out spots showed where the animals had attacked the wood of the tank floor and scooped it out quite distinctly; no similar attack had been made on the tanks containing those fed on laminaria. The experiment lasted from August 25 till Sept. 13, a period of nineteen days. At the end the gonads were removed, examined for sex, and placed at once in absolute alcohol, from which they were taken and analysed some months later in Liverpool.

Early in this experiment a certain number of the echini in each of these three batches succumbed. This result was in no wise due to lack of food, for as great a percentage died in the fed batch as in the corresponding unfed batch. Such mishaps often occur soon after the echini have been taken, for some unknown reason, and then the survivors keep in quite good condition. All the animals alive at the end were active and appeared quite normal and healthy. Out of the fifteen kept in the large tank, nine survived, of which six were males and three were females; of the fifteen fed daily in the fish-hatching tanks, eleven survived, seven females and four males; of the fifteen kept unfed in fish-hatching tanks, eleven also survived, six males and five females.

Experiment IX a. Amount of glycogen in freshly extracted female gonads. This experiment was carried out with a portion, 61.2 g., of the gonads of females of Catch II, freshly extracted with dilute acetic acid. Details as before, amount of glycogen 1.14 g., found on hydrolysis 87.7 per cent. pure = 1.63 per cent. of pure glycogen in fresh gland, or 11.64 per cent. of the dry organic matter.

Experiment IX b. Determinations of (a) lecithides, (b) fats and oils, and (c) glycogen in a portion, 74.55 g., of female gonads of Catch II, placed at once in alcohol. Hot and cold alcoholic extracts were taken and analysed as described, and the glycogen determined in the residue. In percentages of the moist tissue, the following were the results obtained: glycogen, 1.53 per cent.; lecithides, cold alcohol 0.41, hot alcohol 0.25, total = 0.66 per cent.; constants (cold alcohol), sapon. value 210, iodine value 78.0; (hot alcohol), iodine value 91.1. Fats and oils, cold alcohol 0.4, hot alcohol 1.46, total =

1·86; constants (cold alcohol), sapon. value 208, iodine value 180; (hot alcohol), sapon. value 197, iodine value 174.

Percentages expressed on dry organic matter: glycogen, 10·9; lecithides, 4·6; fats and oils, 13·1.

Experiment X a. This was carried out upon a portion, 134·4 g., of the male gonads of Catch II, freshly extracted with dilute acetic acid, etc. Amount of glycogen obtained, 2·66 g. of 81·15 per cent. purity = 1·60 per cent. of glycogen in fresh gland, or 11·3 per cent. of the dry organic matter.

Experiment X b. Determinations of (a) lecithides, (b) fats and oils, and (c) glycogen in a portion, 75·1 g., of male gonads of Catch II, placed at once in alcohol. Details as in Expt. IX b. Results obtained in percentages of moist tissue: glycogen, 1·70; lecithides, cold alcohol, 1·01, hot alcohol 0·26, total = 1·27; constants of lecithides, (cold alcohol), sapon. value 240, iodine value 89, (hot alcohol), sapon. value 197, iodine value 98·2; fats and oils, cold alcohol 0·54, hot alcohol 1·80, total = 2·34; constants of fats and oils, (cold alcohol), sapon. value 206, iodine value 160, (hot alcohol), sapon. value 202, iodine value 181.

Percentages expressed on dry organic matter: glycogen, 11·90; lecithides, 8·89; fats and oils, 16·38.

Attention may again be drawn to the highly unsaturated nature of the fats and oils as shown by the high iodine values, in both male and female glands, and to the high percentages of reserve storage materials throughout. There was also a large amount of organic matter soluble in 66 per cent. alcohol, and a relatively small amount of insoluble protein and tissue debris, but figures for these are not given, as analyses of the portions were not made. The results were similar in this respect to Table II in which the total amount of salts and alcohol soluble extractives are shown in a similar set of experiments.

Experiments XI to XVI. The whole of the glands in each of these experiments were placed at once, after conversion into a soft pulp, in twice their volume of alcohol. Through a misunderstanding they were used for the glycogen determinations after having been extracted with cold alcohol only, so that the figures given below do not represent total amounts of lecithides, or fats and oils. They are comparable amongst each other, however, and show that there is no appreciable diminution of fatty substances as a result of the nineteen-days fast.

Experiments XI and XII refer to the unfed echini in the large tanks; there were three females, weight of gonads 73 g., average 24 g., and six males, weight of gonads 152 g., average, 25 g. Experiments XIII and XIV refer

TABLE II.

Portion of Experiments of Summer 1911. Determinations of Lecithides, Fats and Oils, and Glycogen in Unripe Ova and Spermatozoa of Echinus esculentus. The experiments show results, in animals kept, fed and unfed, during a period of 19 days in laboratory (Port Erin I.O.M.).

Nature of material extracted with cold alcohol	Total weight of extract	Salts, creatine, nitrogenous bases, etc.	Lecithide values			Fat and Oil values			Glycogen
			Total weight	Saponi- fication value	Iodine value	Total weight	Saponi- fication value	Iodine value	
Exps. XI and XII. Unfed animals together in large tank.									
Exp. XI. Ova unfed:									
Weights in 73 g. ...	11.1	8.90	1.16	181	100	0.74	203	186	0.56
Percentages of moist tissue ...	15.2	12.19	1.59	—	—	1.01	—	—	0.77
Percentages of dry organic matter	—	—	11.13	—	—	7.07	—	—	5.39
Exp. XII. Spermatozoa unfed:									
Weights in 152 g. ...	13.55	10.25	1.66	225	125	1.5	230	170	2.41
Percentages of moist tissue ...	8.91	6.74	1.09	—	—	1.0	—	—	1.59
Percentages of dry organic matter	—	—	7.63	—	—	7.0	—	—	11.13
Exps. XIII and XIV. Fed animals in fish-hatching tanks.									
Exp. XIII. Ova fed:									
Weights in 204 g. ...	14.1	11.20	1.88	187	112	0.85	220	180	2.44
Percentages of moist tissue ...	6.9	5.49	0.92	—	—	0.41	—	—	1.20
Percentages of dry organic matter	—	—	6.44	—	—	2.87	—	—	8.40
Exp. XIV. Spermatozoa fed:									
Weights in 118 g. ...	9.30	6.90	1.37	212	118	0.95	209	191	1.20
Percentages of moist tissue ...	7.90	5.85	1.16	—	—	0.80	—	—	1.02
Percentages of dry organic matter	—	—	8.12	—	—	5.60	—	—	7.14
Exps. XV and XVI. Unfed animals in fish-hatching tanks.									
Exp. XV. Ova unfed:									
Weights in 173 g. ...	13.5	9.85	2.11	189	108	1.20	227	166	0.51
Percentages of moist tissue ...	7.8	5.69	1.22	—	—	0.69	—	—	0.30
Percentages of dry organic matter	—	—	8.54	—	—	4.83	—	—	2.10
Exp. XVI. Spermatozoa unfed:									
Weights in 178 g. ...	14.01	10.5	1.20	181	101	1.95	189	152	2.00
Percentages of moist tissue ...	7.88	5.9	0.67	—	—	1.10	—	—	1.13
Percentages of dry organic matter	—	—	4.69	—	—	7.70	—	—	7.91

to echini fed during these 19 days with laminaria, but kept otherwise under strictly similar conditions to those in the two subsequent experiments (Expts. XV and XVI); there were seven females, weight of gonads 204 g., average 29 g., and four males, weight of gonads 118 g., average 29 g. Experiments XV and XVI refer to echini unfed, but otherwise similar to those in Expts. XIII and XIV; there were five females, weight of gonads 173 g., average 34 g., and six males, weight of gonads 178 g., average 29 g. The average total weight of gonads is less in the case of the unfed echini in the large tank, but in those unfed, alongside the fed, in the fish-hatching apparatus no such effect is discernible. In fact the gonads of the unfed females are appreciably heavier than those of the fed females, while the males are practically of equal weight in the two series. The only conclusion valid is that even a nineteen-days period of removal of supplies is ineffectual to cause any real starvation, or appreciably use up reserves.

It would certainly have been expected from the amount of laminaria used by the echini that some increase of weight might have taken place in the fed animals, but it is possible that only a small amount of the nutritive organic matter of the laminaria was actually utilised by the echini. Certainly the faeces still contained a large amount of nutrition for they were swarming with copepoda when examined microscopically. These no doubt came in with the water supply and remained and accumulated upon the faeces of the echini, which formed their food supply.

The analytical results of these six experiments are shown in Table II. The figures obtained in these experiments show no clear evidence of diminution of reserves in the glands as a result of stopping the feeding for nineteen days, for there is still abundance of glycogen and fatty materials.

Experiments of Easter 1912. The echini used in these experiments were taken a week or two after the active sexual period which appears to have been somewhat early in this particular Spring. At least it was difficult to obtain any ripe eggs for fertilisation about the period of this experiment. The catch was made on April 10 and was divided into two lots, the animals of one of which were kept unfed from April 12 till April 21, while those of the other lot were fed on laminaria. The animals were killed at the end by opening the shells, examined for sex, and separated into four heaps, male and female, fed and unfed, respectively. These were immediately fixed by placing in two volumes of alcohol and taken to Liverpool for the analyses, which were conducted as described above; the results are given in Table III. It will be observed that the results do not differ materially from the others. Only percentage figures and the constants are given in the table.

TABLE III.

Organs extracted	Lecithides			Oils			Glycogen
	Percentage of dry organic matter	Saponi- fication value	Iodine value	Percentage of dry organic matter	Saponi- fication value	Iodine value	Percentage of dry organic matter
Exp. XV. Sperm. of fed males, wt. 205 g.							
Cold alcohol	5.1	159.6	86.0	4.1	164.7	150.1	—
Hot alcohol	7.2	176.1	90.1	5.0	122.3	133.4	—
Total	12.3	—	—	9.1	—	—	7.6
Exp. XVI. Ova of fed females, wt. 171 g.							
Cold alcohol ...	2.87	168.1	82.8	3.15	178	134.7	—
Hot alcohol ...	3.92	172.8	89.7	3.92	185.1	129.5	—
Total ...	6.79	—	—	7.07	—	—	6.2
Exp. XVII. Sperm. of unfed males, wt. 62 g.							
Cold alcohol ...	9.10	163.4	94.5	10.43	158	148.6	—
Hot alcohol ...	1.12	174.3	101.0	1.12	112.1	160.6	—
Total ...	10.22	—	—	11.55	—	—	11.1
Exp. XVIII. Ova of unfed females, wt. 160 g.							
Cold alcohol ...	4.08	164.2	88.6	6.08	168.1	142.1	—
Hot alcohol ...	3.76	171.7	96.3	11.68	174.2	118.4	—
Total ...	7.84	—	—	17.76	—	—	9.1

CONCLUSIONS.

1. Both male and female reproductive glands in echinoderms contain large amounts of reserve metabolic products such as glycogen, fats and lecithides.

2. These reserves are only slowly used up, if at all, when the animal is deprived of food.

3. In a reproductive gland richly stored with glycogen, no sugar formation occurs on keeping after death, even in a period of two days.

4. The amount of food consumed is much greater than that required to cover the daily metabolic wants of the animal, and is largely stored in the reproductive glands during the resting period, but it has not yet been possible to trace the conversion of this at the active reproductive season.

5. The fatty constituents of the reproductive organs of the echinoderm are highly unsaturated, and resemble in this respect liver oils.

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REFERENCE.

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