PROCEEDINGS OF

THE ROYAL SOCIETY.

Section B.—Biological Sciences.

The Effect on the Blood Sugar of Fish of Various Conditions including Removal of the Principal Islets (Isletectomy).

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(Received December 1st, 1924.)

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In fishes the sugar of the blood and the glycogen of the liver have been found to vary considerably even in individuals of the same species, and still more so in those of different species. Practically nothing is known definitely of the causes for these variations, and this we consider an important problem to investigate, especially since light might thereby be thrown on the nature of the metabolism of carbohydrates in cold-blooded animals in which the intermediary stages proceed more slowly than in warm-blooded animals. Our interest was aroused in the behaviour of the blood sugar of fishes for other reasons as well. In certain of the bony fishes (Teleostei) the islets of Langerhans exist as definite glands which have come to be known as the "principal islets." Being more or less separated from the pancreatic tissue itself, these can readily be excised, thus making it possible, by examination of the blood sugar, to determine whether a diabetic condition can be induced by isletectomy without removal of any of the pancreas proper. It was of interest also to see whether insulin can affect the blood sugar. Before such investigations could be undertaken it was necessary to know exactly the degree to which the blood sugar of different fishes of the same species may vary independently of such an operation.

Lang and Macleod (1), in confirmation of earlier work by Diamare (2) and

of Bierry and Fandard (3), found that there are usually only traces of sugar in the blood of the Elasmobranchi, such as *Squalus* (dog-fish), but that considerable amounts may occur in the blood of representative Teleostei, such as *Cyprinus* (carp). In the latter fish it was also noted that the amounts may vary from 0·058 to 0·300 per cent. Fandard and Ranc (4) have stated that the blood sugar in fishes is peculiarly susceptible to asphyxial conditions, but so far as we have been able to find they have published no details of their observations. The most important recent work is that of E. L. Scott (5), who has observed the blood sugar in *Mustelis canis*, the fish prior to the observations being kept in traps which were exposed to tide water and, during them, in shallow tanks. The percentage of oxygen was also frequently determined in the water of the tanks. It was found that no blood sugar, or only traces, could be detected in six out of eight individuals, which are described as having been in a subnormal condition. On the other hand, when the fish were asphyxiated by keeping them out of water for varying periods of time, the blood sugar rose rapidly, to attain, in two specimens, a maximum of about 0·240 per cent. after four minutes, followed by a gradual decline, so that a level of 0·032 was reached in one specimen after 15 minutes. The degree of variability in the results is, however, very great, and they do not seem to us to justify the conclusion that the sugar rises within a few minutes and then falls again during the asphyxial period.

One of us (N. A. McC.) along with E. C. Noble had occasion during the summer of 1923 to observe the blood sugar in numerous sea fishes of various species. These were caught by line and the blood was collected either immediately after landing or some time later, the fish being meanwhile kept in sea-water in a bucket or tub.

The blood was obtained by opening the heart through the gills and allowing it to drop into a crucible with oxalate, and the sugar was determined by the Shaffer-Hartmann method. Typical results are shown in Table I.

The most noteworthy fact demonstrated by these determinations is that a considerable degree of variability occurs, not only when the blood of fishes of different species is compared, but also among individuals of the same species, as in the cases of the sculpin and haddock.

In view of the small range of variability which occurs in the blood sugar of man and other warm-blooded animals under normal conditions, it is difficult to understand why this is not also the case in fishes. To determine the causes for this variability seemed a matter of sufficient importance to investigate more thoroughly, especially since, as already mentioned, it was imperative that

Table I.—Blood Sugar of various Teleostei (mg. per cent.).

Skate (*Raja* spec.)	30 38 68 18.
Witch (*Glyptocephalus cynoglossus*) ..	16.
Sculpin (*Myoxocephalus* spec.)	36 61 34 30 34 (Pail for 30 min.) 10 (fresh) 7.
Haddock (*Melanogrammus æglefinus*) ..	36 53 53 28 46 67 64 60 51 60 59 83.
Flounder (*Pseudopleuronectes americanus*)	36 60 53 57 51 60 36.
Eel Pout (*Zoarces anguillaris*)	12.
Sea Raven (*Hemitripterus americanus*)..	186 8 53.
Tom Cod (*Microgadus tomcod*)	22.
Wolf-fish (*Anarhichas lupus*)	14.
Hake (*Merluccius bilinearis*)	72 65 23.
Cod (*Gadus callarias*)	61 70.
Herring (*Clupea harengus*)	61 58.

this should be done before attempting to see whether removal of the principal islets might cause hyperglycæmia.

Methods.

The majority of the observations were made on the sculpin (*Myoxocephalus octodecim spinosus* or *scorpius*) for several reasons. In the first place it is a readily available inshore fish, easy to catch by hand-line, of convenient size and capable of being kept alive for several days in an aquarium, provided the sea water be constantly changed. It is also the only available inshore fish in which the principal islets are sufficiently accessible and isolated, so that they can be excised without any injury to the pancreas.

It was soon observed, however, that the blood sugar in fish taken from the aquarium was, as a rule, decidedly higher than that of freshly-caught fish, even when the water was being frequently changed, so that it became necessary to construct a pen or crib which could be kept in the sea at the depth at which the fish usually occur. For this purpose we constructed a crib, 6 feet square and 3 feet deep, made of a framework of wood (2 × 4 inches) covered by stout netting. By suitable loading with stones the crib was allowed to rest on a ledge of rock, its top, which was hinged to serve as a lid, being 3 feet below the surface of the water at low tide. This precaution was taken so that the crib might not be damaged by wave action, and also so that the temperature of the water might not be influenced on warm sunny days. To transfer fish the

crib was hoisted to the surface and the fish removed by a hand net. After stunning by a blow on the head the pericardial sac was opened and blood removed by a hypodermic syringe, either by inserting the needle into the bulbus arteriosus or by aspirating from the pericardial sac after cutting the bulbus. In the latter case care was taken that no abdominal fluid became mixed with the blood, and frequent comparisons of results obtained on bulbus blood and pericardial blood did not show any difference between the two. From a sculpin of average weight (500 grms.) it is easy to collect from 7 to 10 c.c. of blood directly from the bulbus. Since the blood clots readily, it must be mixed in the syringe with a soluble oxalate. The sugar estimations were made by the Shaffer-Hartmann method and, to control the possibility of dilution, hæmoglobin was measured by the Dare instrument. In all cases the liver was removed and weighed and portions of 5 grms. were usually taken for determination of glycogen by the Pflüger method. In the later observations these portions of liver were preserved in alcohol (95 per cent.) until time could be found to carry through the analyses. It was found by check analyses that this did not interfere with the accuracy of the determinations. The fat was also determined in many of the livers.

Results.

The first observations were made on fish kept in small buckets containing from 3 to 5 litres of sea-water for each specimen. At laboratory temperatures varying between 16° C. and 20° C. the fish under these conditions do not live for longer than 5 hours, and they gradually become very pale from contraction of the chromatophores. Typical results are shown in Table II.

It is clear that the degree of hyperglycæmia which is induced by confinement in stagnant water for similar periods is not the same in different fishes. To determine the factors upon which this variability depends, attention was first of all directed to the glycogen content of the liver, and it will be seen, in the results of July 24, that a parallelism seems to exist between this and the degree of hyperglycæmia induced. Unfortunately glycogen was not determined in most of the other observations of this series, but it was considered that an estimate of its amount might be gained by finding the weight of the liver in relationship to the body weight. Although the amount of fat, as well as the amount of glycogen present in the organ, must affect this ratio, it was thought that the fat would be tolerably constant, so that variability in weight would depend primarily on glycogen. Later results in which glycogen and fat were both determined have not borne out this supposition, so that the liver-

Table II.

Date.	Time in bucket.	Weight of fish.	Weight of liver.	Per cent. of liver-weight to body-weight.	Per cent. of glycogen in liver.	Blood sugar mg. per 100 c.c.
	Hours.	Grms.				
July 24....	5	255	2·95	1·16	0·007	34
	5	227	5·5	2·44	0·09	126
	5	567	22·2	4·00	1·54	163
	5	312	3·7	1·2	0·006	105
July 23....	3½	Large	—	—	5·64	131
	3½	Small	—	—	7·79	168
July 25....	3½	255	6·7	2·63	—	92
	3½	397	20·6	5·26	—	181
July 26....	2½	757	57	7·70	—	232
	2½	425	—	1·42	—	32
July 27....	4¾	482	20·6	4·85	—	110
	4¾	666	22·45	3·33	—	136
July 28....	2½	297	—	—	—	81
	2½	411	12·0	3·00	—	57
August 5	3½	771	59·5	7·7	—	157
	3½	297	25·2	8·3	—	121
August 8	2½ to 3	411	37·6	9·5	—	178
	2½ to 3	482	22·0	4·5	1·12	102
August 9	½	—	16·5	—	—	67
	1	—	2·86	—	—	4
	1½	—	2·91	—	—	126

body-weight ratios are not dependable in estimating the amount of glycogen. It is nevertheless significant that the extent of hyperglycæmia induced by asphyxia often runs parallel with the ratio, as is clearly shown in the results of July 24, 25 and 26 and of August 8. Since such a proportionality did not always exist, however, it was decided that glycogen determinations should be made in all subsequent observations. When we eliminate the experiments in which ratios below 3 occur, it can be seen that the maximum degree of hyperglycæmia is established in about 2½ hours under the conditions of the experiment. The exceptionally low result following asphyxia for one hour in one of the observations of August 9 is difficult to explain, especially since neither the weight of the fish nor the glycogen content of the liver was determined. The very small size of the liver, however, indicates that the organ must have been almost glycogen-free.

In the light of these results it seemed clear that the inequality in the concentrations of blood sugar in line-caught fish recorded by previous investigators, and by McCormick and Noble, was due, in part at least, to variability in the treatment of the fish after catching. Observations were therefore made in which the blood was removed from fish within a minute or two of catching. Typical results are shown in Table III.

Table III.

Date.	How bled.	Weight of fish.	Weight of liver.	Per cent. of liver-weight to body-weight.	Glycogen.	Blood sugar mg. per cent.	H.B. per cent.
					Per cent.		
July 25	Pericardium	411	10·2	2·5	—	48	—
	,,	255	3·95	1·6	—	30	—
	,,	312	4·3	1·4	—	26	—
	,,	354	11·6	3·3	—	24	—
Sept. 10	Pericardium	193	7·8	4·0	—	12	18
	,,	208	8·0	3·8	12·0	7	25
	,,	177·5	2·5	1·5	6·9	8	45
	,,	396	21·0	5·3	8·72	trace	28

There is no relationship between the liver-weight ratio and the blood sugar in these observations. Why the values of July 25 should be so decidedly higher than those of September 10 is impossible to explain, but it is considered probable that these two sets of figures represent the extreme limits between which the blood sugar of normal, fresh-caught sculpin may vary.

The Rates of Development and of Recovery from Asphyxial Hyperglycæmia.

As has already been mentioned, E. L. Scott has stated that in dog-fish removed from the water the blood sugar rises rapidly, to reach a maximum in about 4 minutes and then more gradually declines so as to return nearly to the normal level in 6 minutes. These time intervals seemed to us by far too brief, at least for *Myoxocephalus*, so that the observation was repeated. The fish were caught by line and were then left in air for varying periods, after which they were bled. The results are shown in Table IV.

Although glycogen was not determined in all the specimens, this was done in a sufficient number to indicate that the percentage was practically uniform, the fish being caught at rich feeding grounds. No detectable hyperglycæmia occurred until after about 30 minutes, and even up to 45 minutes it was

Table IV.

Time after catching.	Weight of fish.	Ratio of liver-weight to body-weight.	Glycogen in liver.	Blood sugar mg. per 100 c.c.
Min.	Grm.		Per cent.	
9	312	3·93	—	18
17	553	3·07	—	10
23	680	4·66	—	12
24	283	4·48	5·6	32
32	425	5·23	5·8	43
35	539	4·45	7·26	12
38	468	5·89	5·70	24
49	828	2·57	5·66	40
49	539	5·47	—	57
60	397	6·63	—	70
81	595	5·48	5·32	132
98	368	3·38	4·84	96

insignificant, although all the fish did not react similarly. After this time, however, the blood sugar increased rapidly.

In another series of observations the freshly caught fish were kept in the air, wrapped up in moist towels for 20 minutes and then replaced in frequently changing sea-water in tubs. The results are shown in Table V.

Table V.

—	Time after asphyxiating.	Weight of fish.	Ratio of liver-weight to body-weight.	Glycogen in liver.	Blood sugar (mg. per 100 c.c.).	Hæmoglobin.
		Grms.		Per cent.		Per cent.*
A Aug. 26	Immediately	269	2·9	5·6	109	26
	1 hr.	212	1·2	—	63	47†
	2 hrs.	241	2·3	0·16	86	34
	3 hrs. 20 mins.	241	1·1	trace	43	32
	4 hrs.	312	2·8	—	262	15
	5 hrs.	255	1·2	—	trace	42
	6 hrs. 40 mins.	340	1·9	3·76	163	—
	11 hrs. 30 mins.	241	2·2	trace	trace	13
	22 hrs.	212	2·1	5·3	135	—
B Aug. 29	1½ mins.	312	5·0	0·11	73	34
	1 min.	340	2·4	3·76	91	27
	1 hr. 10 mins.	496	5·0	6·44	164	45
	2 hrs.	354	4·3	10·96	167	28
	4½ hrs.	595	5·2	0·13	135	20
	6 hrs.	425	4·8	8·00	163	20
	7 hrs.	439	3·3	1·76	110	28
	8½ hrs.	439	4·3	2·76	32	35
	9½ hrs	609	3·4	5·6	59	33
	24 hrs.	482	6·4	6·84	125	29

* The blood in all these cases was collected through a hypodermic needle inserted in the bulbus arteriosus.

† Blood very scanty.

In both series of observations hyperglycæmia was evident even in the fish taken immediately after the period of asphyxiation, which lasted 20 minutes. This would seem not to correspond with the results given in Table IV, the apparent difference being due to the fact that in this case the intervals are counted from the time of removal of the fish from the sea. When the times elapsing between removal of the fish from the sea (crib) and placing them in the moist towels are taken into account, 20 minutes must be added in the case of A and 25 minutes in that of B of Table V. That the transference of the fish by net from the crib to a tub during which they are exposed to air for 2 to 3 minutes is sufficient to cause some degree of hyperglycæmia is probable, although we have no observations to prove that such is the case.

In its subsequent behaviour the blood sugar attained a maximum in about 2 to 4 hours after the fish had been replaced in frequently changing water. This is most clearly seen in the observations of series B. The results of series A are irregular, no doubt mainly on account of the fact that there was practically no glycogen in the livers of three of the fish*—namely, in those removed in 2 hours, 3 hours 20 minutes, and 11½ hours respectively. The very low weight ratio in the fish removed in 5 hours indicates that there was little glycogen in the liver. This experiment, perhaps more than any other, shows that a certain percentage of glycogen in the liver is of great importance in determining the behaviour of the blood sugar to such a condition as asphyxia. It is, however, not the only factor, as subsequent observations have shown.

After about 6 hours there is evidence of recovery to the normal level, especially in series B, although this was so slow that considerable hyperglycæmia still remained in 24 hours after the asphyxia. This is of importance in considering the effects of removal of the principal islets.

The hæmoglobin in both the experiments was very high in the fish removed in about one hour after the asphyxia. Its subsequent behaviour in the fish of A was irregular, but in those of B it fell to a subnormal level until the eighth hour, when it recovered to about the normal. For freshly caught *Myoxocephalus* this is about 30 per cent. by the Dare hæmoglobinometer, in which the error of observation is about 5 per cent.

Influence of Frequent Changing of Water and of Temperature on Blood Sugar.

It has not been found possible to keep the fish in aquaria with frequently changing sea-water so as entirely to prevent the development of hyperglycæmia.

* The much greater irregularity of glycogen in the fish of A as compared with those of B may be related to the fact that the former had been in the crib for five days and those of B for only two days before removal for these experiments.

The aquaria used consisted either of average-sized tubs or shallow wooden trays, the sea-water in both cases being delivered from piping connected with a water-tower.

In the first three observations, recorded in Table VI, it is seen that in from $3\frac{1}{2}$ to $5\frac{1}{2}$ hours in a tub the blood sugar stood at not more than half the height which would be expected in stagnant water. Part of this hyperglycæmia is no doubt to be accounted for by the exposure to air in transferring the fish from the sea. After two days, as seen in the observations of September 11, the hyperglycæmia had subsided considerably. Similar results were obtained by placing the fish in shallow water in the flat aquarium. (Observations of September 1 and 5.) Even under the most favourable laboratory conditions, however, one cannot be certain that the blood sugar will remain at the normal level. After a few days under apparently ideal conditions the fish often become decidedly paler, from contraction of the chromatophores, and when this occurs hyperglycæmia is nearly always present, as in the observation of August 25.

Table VI.

Date.	Time in frequently changing water.	Weight of fish.	Per cent. of liver-weight to body-weight.	Per cent. of glycogen in liver.	Amount of glycogen in liver.	Blood sugar mg. per 100 c.c.	Hæmo-globin.
		Grms.			Grms.		Per cent.
July 23	In tub $3\frac{1}{2}$ hours	638	4·16	4·42	1·175	96	—
	In tub $3\frac{1}{2}$ hours	510	3·85	10·16	2·000	64	—
July 24	In tub $5\frac{1}{2}$ hours	—	—	—	—	77	—
Sept. 1	In aquarium several days	255	2·4	—	—	32	—
Sept. 5	In aquarium $7\frac{1}{4}$ hours	215	2·55	1·48	0·080	36	18
	In aquarium $7\frac{1}{4}$ hours	204	2·05	1·68	0·71	43	29
Sept. 11	In tub 2 days	248	3·2	—	—	56	29*
	In tub 2 days	532	6·0	—	—	20	32
	In tub 2 days	336	4·8	—	—	20	37
Aug. 25	In aquarium 3 days	241	4·7	—	—	316	—

* Liver cirrhotic.

The influence of temperature on the development of hyperglycæmia in stagnant water was studied early in the investigation before the necessity of considering the glycogen reserves had been demonstrated. Such results as were obtained show clearly that cooling delays the onset of the hyperglycæmia. Thus, in two fish kept in a tub at 17°·2 C. for 4 hours the blood sugars rose to 0·116 and 0·274 per cent., whereas in two others kept under similar conditions, except that the temperature was 9°·5 C. (by placing the bucket in the ice house), the blood sugars were 0·095 and 0·100 per cent.

In another similar observation the blood sugars in two fish kept at 17°·5 C. were 0·157 and 0·081 per cent., and in two fish at 9°·7 C. they were 0·078 and 0·080. A decided retarding influence of cold is evident from these results.

Behaviour of Blood Sugar of Fish kept in Crib.

In light of the preceding observations it is to be expected that the hyperglycæmia due to hooking and handling the fish previous to placing them in the crib will take some time to subside. It is important to determine how long this is, not only so that the influence on the blood sugar of various experimental conditions may be determined, but also because the persistence of hyperglycæmia may be related to the subnormal general condition of salt-water fish after returning them to the sea, as in the operation of tagging. The fish are thought by those who have had large experience in this work, which is done for the purpose of studying the problem of migration, to be below par for some time and so to be liable to fall a prey to their enemies.

Fish removed by hand-net were bled at daily intervals after placing them in the crib. In the first observations the blood sugar alone was determined, with the following results :—

Fish placed in pen on July 19—

Removed 2 days later, the blood sugars in two fish were 63 and 73 mg. per cent.

Removed 3 days later, the blood sugars in two fish were 40 and 43 mg. per cent.

Removed 4 days later, the blood sugars in two fish were 99 and 75 mg. per cent.

Removed 5 days later, the blood sugars in two fish were 18 and 24 mg. per cent.

The unexpected persistence of the hyperglycæmia up to the fourth day made it advisable to repeat the observations, especially since no measurement had been made in the above, of the glycogen content of the liver. This is done with the results shown in Table VII.

It is seen, from the third day on, that the blood sugar of the crib fish in 11 out of 13 observations did not exceed that found in freshly caught fish. Occasionally, however, as in one of the fish removed on the fourth and in another removed on the ninth day, somewhat higher values, viz., 53 and 56, were found. These are not associated with an excess of glycogen in the liver and cannot be accounted for in any other way. That they may occur must be borne in mind,

Table VII.

Date.	Days in pen.	Weight of fish.	Per cent. of liver-weight to body-weight.	Per cent. of glycogen in liver.	Amount of glycogen in liver.	Blood Sugar.	Hæmoglobin.
		Grms.			Grms.	Per cent.	Per cent
Aug. 29	1	241	3·3	4·96	0·400	53	30
Sept. 9	1	—	—	—	—	36	30
	1	350	—	4·1	—	205	31
Sept. 10	2	366	4·3	4·7	0·74	20	30
Aug. 30	2	241	3·14	—	—	93	35
Sept. 1	3	297	3·0	3·64	0·33	trace	—
	3	269	5·2	—	—	20	—
Sept. 10	4	213	3·6	3·6	0·28	trace	—
July 28	4	326	3·4	0·39	0·04	30	—
	4	340	4·5	—	—	38	—
	4	638	5·0	—	—	24	—
	4	312	2·4	—	—	53	—
Sept. 12	4	162·5	1·5	—	—	trace	—
	4	368	4·2	—	—	32	—
Aug. 6	6	283	7·4	—	—	36	—
	6	255	7·4	—	—	24	—
Aug. 9	'9	—	—	—	—	56	—
Sept. 9	11	—	—	2·08	0·20	24	30

especially in investigating the effect of experimental conditions, such as isletectomy, on the blood sugar.

When a *prolonged period of asphyxia* (20 *minutes in air*) *precedes placing the fish in the crib* the hyperglycæmia may persist for much longer than when this is only of short duration, such as was the case in the observation of Table VII. To serve as controls for observations in which the islets were removed, it was necessary to ascertain for how long the hyperglycæmia would last in fish exposed to the air for the same time as the operation of isletectomy. The results are shown in Table VIII and also charted in the diagram on p. 20.

These observations show that the hyperglycæmia may persist even until five days after the asphyxiation (Nos. 287 and 158). After this period, however, none of the blood sugars went beyond the upper limit of 35 mg. per cent. observed in fresh-caught fish. The marked hyperglycæmia seen in fish No. 287 is impossible to account for, there being practically no glycogen in the liver. It is noteworthy that there was also unusually little fat and that the hæmoglobin was only 18 per cent.

The Relationship of Glycogen to Asphyxial Hyperglycæmia.

Although it is clear that asphyxia, due either to removal of the fish from water or to inadequate replacement of the latter, is a potent cause of hyper-

Table VIII.—Effect of 15 to 20 minutes asphyxia on subsequent behaviour of Blood Sugar.

No.	Date of asphyxiation.	Days after asphyxiation.	Blood sugar per cent.	Hæmoglobin.	Fish weight.	Per cent. of liver-weight to body-weight.	Glycogen in liver	Fat in liver.	Remarks.
			Mgs.	Per cent.	Grms.		Per cent.	Per cent.	
261	August 19	1	201	30	312	2·3	2·3	—	
132	,, 30	2	0·045	—	—	—	—	—	
271	,, 20	3	127	—	425	2·7	4·24	—	Asphyxiated about 20 m. by placing in towel.
264	,, 19	4	53	33	382·5	3·7	1·60	—	
287	,, 20	5	367	18	226	2·0	—	14·3	
158	,, 30	5	67	—	348	2·3	1·36	—	
166	September 4	9	trace	—	204	2·1	3·3	5·68	
197	August 30	10	34	28	235	2·2	0·24	—	
136	,, 30	10	32	30	250	2·2	6·36	26·8	
168	September 4	10	trace	27	231	2·5	0·09	12·8	
148	,, 4	10	36	30	408	3·1	0·88	—	
142	August 30	13	28	16	279	3·1	0·054	27·6	
276	,, 20	24	20	—	—	—	—	32·7	

glycæmia, there are evidently certain other factors which may have the same effect or which may greatly influence that of asphyxia. We thought at first that these might be dependent upon whether or not the fish had recently been feeding, and to put this possibility to the test we divided the crib into two compartments by netting and placed large amounts of food in one of them. It was found unreliable to determine the possible influence of feeding in this way, mainly because only certain of the fish in the " fed " compartment would take the food. There was also the risk that certain of the supposedly starved fish would get some of the food by its being washed by tide currents through the meshes of the dividing net. It was partly to control this factor that it was decided to determine the glycogen content of the livers. A less laborious method would have been to examine the stomach contents, but it was found in the cases in which this was done that not infrequently the stomach was empty when it seemed certain that food had been taken within a day or so. It was mainly for this reason that it was considered that a much safer criterion of the nutritive condition of the fish would be the glycogen content of the liver.

The relationship between the degree of asphyxial hyperglycæmia and the liver glycogen has already been referred to in connection with the results in Table II. It is also seen very distinctly in Table V, in which asphyxia failed to cause any hyperglycæmia in two fish in the livers of which no glycogen could be detected and only a very slight degree of hyperglycæmia in another. But there are evidently wide limits between which the amount of glycogen in the liver does not influence the behaviour of the blood sugar following asphyxia. Thus, when the total amount of the glycogen exceeds 0·05 grm. there does not appear to be any relationship. So long as the glycogen stands at a certain fairly low level, its mobilization as sugar in response to the establishment of an asphyxial condition is maximal, and the only influence which it probably has is on the duration of the hyperglycæmia. Since this might subside for another reason, however (namely, the disappearance of the asphyxial condition itself), it is impossible to put the suggestion to the test. The ratio of liver-weight to body-weight has not proved to be of much value as a guide either to the amount of glycogen or the sensitiveness of the blood sugar to change. It does, however, indicate in a rough way whether food has recently been assimilated, and it will be seen, as in the observations following asphyxia (Table VIII), that it is usually below 3 in fish that have been kept in the crib for any length of time.

The failure to demonstrate any close relationship between the extent and

duration of asphyxial hyperglycæmia and the glycogen content of the liver raises the question whether the extra sugar which appears in the blood in such conditions may be derived, in part at least, from "masked" carbohydrates in the blood itself, such, for example, as gluco-proteins, lower polysaccharides, or substances of a glucosidal nature. To investigate this possibility we have measured the change in reducing power caused by heating the blood in the presence of various concentrations of mineral acid (HCl). The following are typical results :—

I. *July* 12 :—

(1) Mixed blood from 5 fish contained 0·024 per cent. glucose.

(2) Protein-free filtrate heated on boiling water bath with 1 drop HCl (conc.) contained 0·036 per cent. glucose.

(3) Blood heated in presence of 2 per cent. HCl contained 0·075 per cent. glucose.

II. *July* 14 :—	I.	II.	III.	
Fresh blood from each of 3 fish kept some time in tub contained	0·059	0·058	0·191	per cent. glucose.
Same blood in each case but after heating for 1 hour in presence of 0·1 NHCl contained	0·101	0·099	0·199	„ „

III. In the following cases the filtrates by the Shaffer-Hartmann method were hydrolysed in the presence of 2 per cent. HCl :—

Mg. Glucose per 100 *c.c. Blood.*

	Before hydrolysis.	After hydrolysis.
Skate	30	40
Sculpin	34	22
	34	42
	61	104
Haddock	46	65
	83	86
Flounder	36	51
	57	64
Sea Raven	53	60
Wolf-fish	14	36
Cod	70	105

It is evident that a considerable amount of masked carbohydrate occurs in fish blood, as evidenced by the increase in reducing power which can be caused by hydrolysis. Generally, this is much more marked when the blood itself is hydrolysed than when the protein-free filtrate as obtained in the Shaffer-Hartmann method is employed. This would seem to indicate that the masked carbohydrate exists in some colloidal form which is precipitated along with the proteins, and to judge from the reducing power after hydrolysis it is possible, in sculpin blood at least, to account for most of the sugar which appears as a result of asphyxia as coming from it. It was not possible to carry these investigations to completion, but it may be said that we have obtained other evidence which supports the view that the " sugar " in the blood of fishes is in certain particulars different from that of mammals.

Observations were also made on the process of *glycolysis*. It has been found that this does not occur within several hours in the oxalated blood of the sculpin kept at room temperature. Thus :—

Blood removed (on August 29) at 3 : 05 from an asphyxiated fish contained 0·135 per cent. sugar. After standing 6½ hours, the test-tube being meanwhile frequently shaken, it contained 0·131 per cent.

Blood from another asphyxiated fish removed at 11 : 38 contained 0·164 per cent. sugar. After 10 hours without shaking it contained 0·167 per cent.

Mixed blood removed from several freshly caught fish contained 0·024 per cent. sugar. After standing 1 hour 10 minutes it contained 0·024 per cent. After 3 hours 10 minutes 0·024, and after 21 hours 0·036 per cent.

It is, of course, possible that the oxalate may have interfered with the glycolytic process, since it has been found by one of us to have this effect on glycolysis in mammalian blood (11).

Having determined, as far as was possible in the time available, the conditions under which changes occur in the blood sugar when the fish are kept for the same time and handled to the same extent as would be necessary in investigating the influence on it of various experimental conditions, the effects of injections of epinephrin and insulin and of removal of the principal islets were investigated.

The Effect of Epinephrin.

There is no more certain way for producing hyperglycæmia in mammals than by the subcutaneous injection of epinephrin (adrenalin chloride). It was therefore considered important to see whether the same is true in fish.

Thirty minutes after removal from the crib, 1 c.c. adrenalin chloride was injected into each of four fish, which were then kept in running water. Two

fish were placed under the same conditions, but were not given epinephrin. The results are shown in Table IX.

Table IX.

Time after epinephrin.	Weight of fish.	Per cent. of liver-weight to body-weight.	Per cent. of glycogen in liver.	Glycogen of liver.	Blood sugar.	Hæmoglobin.
	Grms.			Grms.	Per cent.	
55 mins.	324	4·4	11·2	1·60	101	28
1 hr. 45 mins.	240	2·08	0·5	0·025	173	—
4 hrs. 15 mins.	255	—	1·3	—	188	—
7 hrs. 15 mins.	412	2·9	0·1	trace	88	28
Control 1	215	2·5	1·48	0·082	36	18
Control 2	204	2·05	1·68	0·071	43	29

The blood sugar rose rapidly, gaining a maximum in 1 to 4 hours and then declining. Shortly after the injections the fish became excessively pale from contraction of the chromatophores, and since there could be no doubt that the blood sugar responds in the same way as in mammals, the experiment was not repeated.

The Effect of Insulin.

At an early stage in the investigation of the effect of insulin on the blood sugar of normal warm-blooded animals a few observations were made by us to ascertain its action on cold-blooded animals. Insulin was injected into the dorsal lymph sac of frogs, and these were kept for several days at average room temperature. Even when relatively massive doses were injected the frogs were not observed to develop any symptoms, although they were kept in the laboratory until the fourth day following the injection. A few months later A. Krogh informed us that he had found that when the frogs were kept after injection for longer periods of time symptoms supervened which were comparable with those induced by insulin in mammals. The frogs became hyperexcitable, unable to maintain their equilibrium, and they often showed convulsive seizures, the symptoms being relieved by glucose. A repetition of the observations in this laboratory in the spring of 1923 by J. M. D. Olmsted confirmed these findings, and it was further observed that the onset of the symptoms could be accelerated by warming the injected frogs. Attempts to determine whether the blood sugar became gradually lowered preceding the onset of the symptoms failed, because of the fact that in frogs kept for some time under laboratory

conditions this is very low (about 20 mg. per 100 c.c.). Having noted that the blood sugar rises considerably in frogs when they are kept for two days at 28° C., Olmsted found that insulin does not cause a lowering of this abnormally high blood sugar. Observations on the effect of insulin on frogs were made later by Julian Huxley and Fulton (7), who found that a period lasting several days supervened between the injection and the appearance of symptoms. This could be shortened by warming the frogs; thus it took from five to six days for symptoms to appear when the animals were kept at 7° C., whereas it took only 24–27 hours in the case of those kept at 25° C. Two other important facts were also noted, namely, that the time of incidence of symptoms bore no relationship to the dose of insulin within wide limits, and that frogs kept cool for some days after injection quickly developed symptoms when subsequently warmed. In the summer months of 1923 Olmsted continued his studies on the effect of insulin on cold-blooded animals, using fresh-water catfish (*Ameiurus nebulosus*) and described the development of peculiar symptoms supervening in about two days following the injections, the fish being meanwhile kept at room temperature. These could be only temporarily relieved by injections of glucose. One of us (N. A. McC.) working at St. Andrews also observed peculiar symptoms to develop in the Sculpin (*Myoxocephalus*) and Sea Raven (*Hemitripterus*) several days after the injection of insulin.

Assuming that the symptoms observed in these cases are related to a fall in blood sugar to a certain low level, as is the case in mammals, it would appear that insulin causes hypoglycæmia in cold-blooded animals just as it does in mammals. However, this is not inevitably the case, and as a matter of fact Noble and Macleod (8) have been unable to demonstrate any fall in blood sugar as a result of the administration of insulin to turtles. Houssay, Sorddelli and Mazzacco (9) and later Houssay and Rietti (10) have also published brief statements of investigations of the effects of insulin on different cold-blooded animals, including frogs, toads, turtles, snakes and fish. In the latter no special symptoms were observed to follow the injection of large amounts of insulin.

Apparently, therefore, nothing definite is known concerning the effect of insulin on the blood sugar in fish, and it was decided to study this question in sculpin, with the results shown in Table X.

Since the blood sugar in the normal sculpin is not infrequently practically at zero it is not possible to demonstrate any effect of insulin on it. This was attempted in one of the experiments of September 14, in which two fish were injected with 10–20 u. of insulin a few days before removing them from the crib, and then daily for a further period of four days, during which they were

Table X.—Insulin.

Date.	Condition.	Weight of fish.	Per cent. of liver-weight to body-weight.	Glycogen in liver.	Blood sugar.	Hæmo-globin.
		Grms.		Grms.	Per cent.	
Sept. 14....	10 u.–20 u. daily for 4 days; also previously in pen	255·0	1·8	none	16	33*
		461·5	4·6	—	8	27
Sept. 14....	10 u.–20 u. daily for several days, then 10 u., then asphyxia for 3 hrs.	472	4·6	—	72	—
		439	3·0	0·486	123	30
		526·5	3·1	0·107	60	16
		375	4·0	0·195	81	30
Sept. 13....	5 u. insulin at 9 a.m., asphyxia 10 : 30–12 : 30	628·5	6·1	1·500	63	—
		489·5	5·7	0·400	101	—
Sept. 13....	10 u. insulin several days previously in crib. Then 10 u. a day for 3 days in lab. Asphyxiated 11 : 15–1 : 15, insulin given 11 : 30	389·5	—	—	80	—
		537	3·2	trace	70	20
Aug. 5	10 u. on day previous asphyxia, 3½ hours	397	33	—	121	—
		439	33	—	72	—
Aug. 6	10 u. 2 days previously asphyxia, 2½–3½ hours	269	—	—	56	—
		595	—	—	153	—
		340	—	—	96	—
Aug. 8	10 u. 4 days previously asphyxia, 3 hours	439	—	—	150	—
		539	—	0·03	12	—†

* Also given insulin few hours before bleeding.
† Liver cirrhosis.

kept in rapidly changing water in the laboratory. The blood sugars of 16 and 8 mg. per cent. that were found are at the lower limits of the normal range.

To demonstrate any effect of insulin it was necessary to subject the fish to some condition which would cause hyperglycæmia and then to see whether insulin could influence it. Asphyxia was chosen as the agency causing hyperglycæmia, and it will be seen from Table X that insulin has only a slight and uncertain influence on it. Thus, if we compare the observations of Table X with those of Table II (omitting in both cases fish with liver-weight ratios of less than 2·5) it is seen, in one of the experiments of September 13, that asphyxia for two hours raised the blood sugar to 63 and 101 mg. when the insulin was injected two hours previously, and in another observation on the same day, when the insulin had been given on several days previously, to 70 and 80 mg.

per cent. These figures do not differ essentially from those obtained on normal fish asphyxiated for two hours, viz., 232, 34, 81 and 57 (Table II). When the comparisons are made for fish asphyxiated for from three to three and a half hours, we find in the insulin-injected fish (all injected frequently during several days preceding the asphyxia) 72, 123, 60, 81, 121, 72, 56, 153, 96 and 150, average 98·4 mg. per cent., as compared with 131, 168, 92, 181, 110, 136, 157, 121, average 137·0 mg. per cent. in uninjected fish. If we base our conclusion on the averages of the results there is some evidence that insulin hinders the development of asphyxial hyperglycæmia, and this is supported when we compare the maximum and minimum values in the two groups; thus, in uninjected fish there are max. 168 and 92, and in injected fish 153 and 56 mg. per cent. The hypoglycæmic action of insulin is comparatively feeble in fish.

The Effect of Removal of the Principal Islets (Isletectomy).

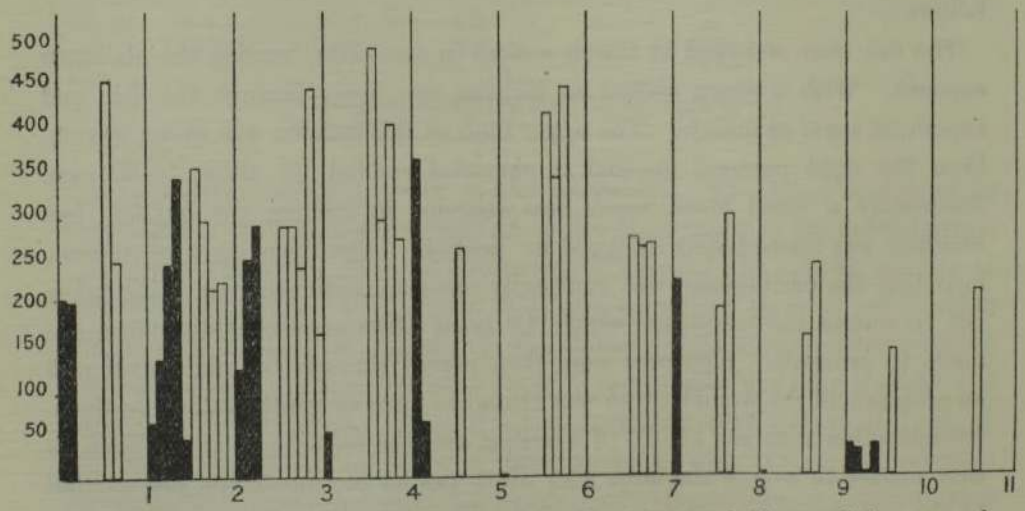
The sculpin is probably the only readily available inshore fish in which the principal islets are sufficiently isolated from the pancreas to make it possible to excise them, leaving the pancreas itself intact. By microscopic examination of sections of the pancreatic tissue in this fish both Slater Jackson and W. C. M. Scott have reported occasional small islets, but when their total mass is compared with the mass of tissue represented in the principal islets it must be almost insignificant. The operation of isletectomy was performed as follows:—

The fish were wrapped in towels soaked in sea-water, leaving the abdomen exposed. With a sharp scalpel an incision was made through the skin and superficial layer of muscle. The upper limit of this incision was about 10 mm. from the right pectoral fin, and it extended caudad for about 50–60 mm. Frequently a small blood vessel was wounded in making the incision, but bleeding was immediately stopped by ligation. The deep layer of muscle, including the peritoneum, was cautiously opened, particular care being taken not to wound the intestines, which, by being often somewhat distended, are liable to protrude. Ligatures were then passed through the abdominal wall on each side of the wound which was caused to open by gently pulling on them. By retraction with the handle of a scalpel and the little finger it was then an easy matter to expose the large islet which lies on the duodenum just caudad to the pyloric cæca. It was picked up by a fine dissecting forceps, a thin ligature tied around its base and the gland excised. Sometimes several small islets lie in a cluster in the immediate neighbourhood of the large one. These, when present, were also mass-ligated and excised. The intestines

were then further retracted so as to expose the spleen and the other large principal islet which lies at its inferior pole. This was also picked up in a forceps, mass-ligated and excised. In applying the ligatures at both islets care had to be taken not to include any of the larger vessels, so as to avoid congestion in the mesentery. The wound in the abdominal wall was closed by two layers of discontinuous sutures, one through the muscle and peritoneum and the other through the skin. Out of a total of over 50 fish operated on and subsequently recovered from the crib, opening of the wound after placing the fish back in water was observed in only two. The entire operation occupied about 15 minutes, and the fish were returned to the crib as soon as possible so that the conditions for their recovery from the effects of asphyxia might be as favourable as possible.

As already mentioned, other fish were used as controls, some of them being merely asphyxiated by wrapping in wet towels for the same time as that required for the operation of isletectomy, but on others the operation was performed in all its details except the actual removal of the islets.

Fish were removed from the crib at varying intervals following the isletectomy, or control operation, and the sugar determined, with the results shown in Table XI, and in the accompanying chart, where the blood sugar levels are given in vertical columns and the observations are grouped according to the number of days elapsing between the operation and the removal of blood.



White columns represent percentage of blood sugar in specimens of *Myxocephalus* removed on various days following isletectomy. Black columns represent percentage of blood sugar in control fish. Percentage of blood sugar is given vertically. Days after isletectomy are indicated on base-line.

The white columns give the results for isletectomised fish, the black columns for the operated controls. Further details with regard to glycogen and fat in the liver, weight of fish, etc., are given in Table XI. In Table XII are given the operated controls, the asphyxia controls being shown in Table VIII.

In the results for the five days following the operation the difference between the two groups is not conspicuous, although the average for the isletectomised fish on each of the days is higher than the controls. Thus, the sugar in mg. per 100 c.c. blood is as follows :—

Controls.	*Isletectomised.*
1st day—*202*, 201, Av. 201·5	1st day—456, 250, Av. 353
2nd day—63, *135*, *242*, *343*, *203*, 45. Av. 172	2nd day—359, 297, 220, 229, Av. 276
3rd day—127, *250*, *292*, Av. 223	3rd day—290, 290, 243, 446, Av. 317
4th day—53, Av. 53	4th day—490, 298, 406, 275, Av. 367
5th day—367, 67, Av. 217	5th day—263, Av. 263

The high level of the controls up to five days is no doubt mainly dependent on asphyxia, but it is possible that the actual opening of the abdomen, the slight hæmorrhage and the irritation of the stitches might also tend to cause hyperglycæmia. It was to allow for these possible factors that the controls of Table XII were run. The value of 0·239 per cent. observed in one of the operated controls, removed on the eighth day following the replacement of the fish in the crib, is very difficult to account for. There are certain details of this observation, however, which are noteworthy. In the first place, the blood was very thin and contained only 15 per cent. of hæmoglobin. It is a curious fact, that in the vast majority of all the fish examined, in which the hæmoglobin was below 20 per cent., high sugar levels were found to occur. It is also important to note that this fish, after removal from the pen, was probably kept for more than an hour before being bled—at least the notes indicate that the first fish to be used on this day was bled at 9.30 a.m. whereas this one was not bled until 10.45 a.m. This indicates the possibility that asphyxial hyperglycæmia may have been induced and so account for the high blood sugar.

After the fourth day following the operation the control asphyxiated fish (*cf.* Table VIII) were unfortunately not so numerous as those that were isletectomised, the reason being that owing to an accident many of the former escaped from the crib. There were nine of these controls with blood sugars that were well within the normal range, but there was one fish, on the fifth day, in which 367 mg. was found. It is a curious coincidence that this fish was bled on the same day as No. 260, and it is possible that, after removal from the crib, both were inadvertently kept for too long in stagnant water. In any

Table XI.

Isletectomies.

No.	Date of isletectomy.	Days after isletectomy.	Blood sugar per cent.	Hæmoglobin.	Fish weight.	Per cent. of liver-weight to body-weight.	Glycogen in liver.	Fat in liver.	Remarks.
			Mgs.	Per cent.	Grms.		Per cent.	Per cent.	
101	August 15	1	456	—	255	2·9	—	—	*P.M.* Small piece splenic islet remaining possibly not tied.
129	,, 15	1	250	—	184	2·2	5·44	—	*P.M.* Wound open, intestines escaped.
109	,, 14	2	359	30	—	—	5·92	—	
134	,, 14	2	297	55	212	2·3	6·64	—	
102	,, 16	2	220	20	142	2·4	0·70	—	*P.M.* Portion splenic islet left.
215	,, 21	2	229	30	283	3·6	5·7	—	*P.M.* Small mesenteric islet left.
145	September 2	2	113	30	387	4·8	4·9	—	Islet in mesentery.
104	August 15	3	290	35	—	—	0·15	—	
122	,, 15	3	290	−10	255	3·2	0·06	—	
192	,, 15	3	243	22	567	4·7	0·8	—	*P.M.* Two small islets left.
193	,, 15	3	446	27	269	3·6	2·50	—	
277	,, 22	3	167	10	425	2·5	0·170	31·2	
247	,, 19	4	298	30	—	—	0·24	—	
270	,, 19	4	406	−10	439	2·8	3·12	—	H.B. Below 10 per cent.
283	,, 21	4	275	23	241	1·3	0·016	—	
258	,, 21	4	490*	28	454	3·7	3·44	—	* Minimum.
139	,, 15	5	263	30	312	2·3	0·14	—	
242	,, 19	6	343	28	326	2·8	1·26	20·24	
185	,, 29	6	418	30	387	4·7	—	27·12	
159	September 2	6	450	18	430	4·8	2·30	—	
117	August 29	7	276	13	509	3·7	—	42·0	
152	September 4	7	266	23	521	4·0	—	—	
183	,, 4	7	270	25	610	4·0	—	—	
151	August 30	8	195	35	511	4·2	0·19	43·0	
165	September 4	8	302	8	402	3·1	0·14	29·4	
157	August 29	9	166	18	653	4·4	1·34	37·4	
118	,, 31	9	250	−10	208	3·6	—	—	
198	,, 31	10	148	—	286	4·0	0·3	21·8	Operation difficult.
146	,, 31	11	215	25	476	5·4	0·33	35·3	
140	September 2	11	145	25	381	5·5	0·56	54·0	Fluid in abdomen, may be mixed with blood.
259	August 19	16	trace	15	231	2·7	0·54	19·4	

Table XII.

Controls (*Operated*).

No.	Date of operation.	Days after operation.	Blood sugar per cent.	Hæmoglobin.	Fish weight.	Per cent. of liver-weight to body-weight.	Glycogen in liver.	Fat in liver.	Remarks.
			Mgs.	Per cent.	Grms.		Per cent.	Per cent.	
188	August 17	1	202	20	283	2·4	6·24	—	
189	,, 16	2	135	26	283	2·9	8·00	—	
196	,, 17	2	242	26	553	5·6	13·00	—	
263	,, 18	2	343	20	354	6·1	9·7	—	
245	,, 18	2	203	38	198	2·5	0·77	—	
222	,, 21	2	63	28	453	3·4	7·6	—	One islet removed.
187	,, 17	3	250	30	425	5·2	12·24	—	
121	September 6	3	292	25	231	2·7	3·56	15·5	
130	,, 6	6	trace	—	215	2·3	0·19	15·2	
260	August 17	8	239	15	312	2·9	2·9	—	Fish almost lifeless when operated on.

case the blood sugar in the highest of the controls after the fifth day is decidedly lower than the majority of the isletectomised fish. Of the latter, there are in all fourteen in which the times of survival are known, and in all of them, with one exception, hyperglycæmia of marked degree existed. This exception occurred in the case of a fish that survived the operation for sixteen days, when it was found with the abdominal wound considerably opened and the abdominal cavity filled with water, so that the low percentage of hæmoglobin (fifteen) may have been due to accidental dilution of the blood which was removed from the pericardium after puncturing the bulbus.

It will be observed, especially on examining the chart, that the hyperglycæmia became less and less marked as the interval following the isletectomy became greater. This does not appear to be related to exhaustion of the glycogen reserves.

Besides the foregoing there were three isletectomised fish which lost their tags. One of these found on September 8, which was four days since the last isletectomy, gave a blood sugar of 347 mg. per cent., and another removed on September 12, which was eight days, gave 440 mg. It is possible that both of these had been isletectomised for longer periods. Another untagged fish was removed on August 23, but since fish were being operated upon every day about this period it is impossible even to guess at the date of operation. This fish was taken because it was found in a moribund state in the pen with the abdominal wound partly open and much corrosion of the skin. After removal of the very watery blood from the bulbus, examination of the abdominal contents revealed rupture of the bile duct and evidence of peritonitis.

Taking the results as a whole, there can be no doubt that marked hyperglycæmia was set up as a result of the removal of the principal islets. That exceptional results were occasionally obtained, especially among the controls, does not seriously detract from this conclusion, since it has been shown in the first part of this paper that there yet remains, apart from asphyxia, some unknown factor which influences the blood sugar level. It is possible that this may be muscular effort. The great majority of sculpin, as far as could be observed, were seen to remain practically stationary when the crib was undisturbed and only to move when excited by the landing net or, for a few minutes, after hoisting up the crib. Occasionally a fish would be seen to be constantly swimming about, however, and it is possible that in such a fish the blood sugar was above normal. We propose to investigate the possibility further. Meanwhile, it is of interest to remark that the normal blood sugar of brook trout has been found by Noble and one of us to be much higher than in the

sculpin (0·100 mg. per cent.), these being much more active fish since they have to be constantly swimming against the stream.

The Effect of Isletectomy on the Amounts of Glycogen and Fat in the Liver.

Further evidence that the islets exercise a profound control over the metabolic processes is furnished by comparison of the amounts of glycogen and fat in the liver. Both substances were determined in a considerable number of cases, and for this purpose the pieces of liver after removal from the alcohol were mopped with filter paper and divided into two equal portions—one for determination of glycogen and the other for determination of fat. For this purpose the latter portion was heated on the boiling water bath, with saturated KOH solution and one half of the alcohol in which the liver had been preserved added, after the liver had become dissolved. In some cases the mistake was made of adding all of the preserving alcohol, but the error thus incurred cannot have been very great. The Leathes' modification of the Kumagawa-Suto process was used for determining the fat. The following results for fat and glycogen from fish kept for at least seven days after isletectomy are regrouped (in order of the amount of fat) so as to show the influence of isletectomy :—

Isletectomy.			*Controls.*		
No.	Fat.	Glycogen.	No.	Fat.	Glycogen.
259	19·4*	0·54	166	5·7	3·30
198	21·8	0·30	168	12·8*	0·10
165	29·4*	0·14	287	14·3	trace
146	35·3	0·30	130	15·2	0·19
157	37·4	1·34	136	26·8	6·36
117	42·0	trace	142	27·6	0·05
151	43·0*	0·20	176	32·7*	0·24
140	54·0*	0·56			
Av.	35·3	0·42	—	18·6	1·46

* All of preserving alcohol added during saponification.

There is decidedly more fat and somewhat less glycogen in the diabetic as compared with the normal liver. Since it may be objected that the data are not sufficiently numerous to justify this conclusion, we add for comparison with those of the diabetic fish, results obtained on fish which were used in various of the experiments on asphyxia, etc., and which have been recorded in the first portion of this paper. These are arranged in the order of the amounts of fat found present.

Date.	Fat.	Glycogen.	Liver-body-weight ratio.
September 5	15·8*	11·2	4·4
August 26	17·4*	5·6	2·9
September 10	18·0*	12·0	3·8
August 25	22·2	7·6	4·7
August 29	22·6*	3·8	2·4
September 10	24·4	4·7	4·3
August 29	52·5	0·1	5·0
September 5	29·2*	1·5	2·6
August 29	29·7*	5·0	3·3
August 26	30·0*	5·3	2·1
August 29	31·7	2·8	4·3
September 14	36·5*	1·3	4·0
August 29	38*0	6·4	5·0
Average	26·23	5·17	

It will be seen that five out of nine of the fat values for the isletectomised fish exceed the maximum for the control fish, and six of them exceed the average for those of the last table. When it is remembered that the isletectomised fish were without food for at least six days, the contrast becomes very marked. The differences in the amounts of glycogen in the two tables are also marked, but in this case it is difficult to say to what extent isletectomy is really responsible, since several of the fish used as "controls" gave very low values. Incidentally it will be observed in the results from normal fish that there is no evident relationship between the amounts of fat and glycogen, or between these and the weight ratios. In a general way, when excess of glycogen is present there is much less fat than usual, but high percentages of both may exist side by side. When subnormal amounts of both are present fasting is the probable cause.

These results afford further evidence that the source of insulin in the animal body is the islet tissue. When this new evidence is considered along with that furnished by the fact that extracts of the principal islets contain insulin in quantities far in excess of those extractable from other tissues, including the practically islet-free pancreas of Teleostei (*Myoxocephalus*, *Lophius*) the evidence for the hypothesis that the islets are the source of insulin in the animal body would seem to be complete. That it should be possible to prepare from organs and tissues, other than the pancreas and the principal islets, extracts having insulin-like effects on normal rabbits is probably to be interpreted as indicating the storage in them of insulin transported by the blood. In any case, extra-pancreatic insulin cannot be of significance in the regulation of the metabolism of the carbohydrates, since this completely breaks down when the

pancreas is removed in mammals, but not when any other organ or tissue is removed. If it be the case that administration of insulin from other sources than the pancreas or principal islets can remove the diabetic symptoms, we must conclude that the traces of insulin said to be present in the tissues of the depancreatised animal are not available in its metabolism, possibly because they are combined with some substance so as to produce an inert compound, such as has been demonstrated by Epstein and Rosenthal (14) to be formed between insulin and trypsin or pepsin (15). It was not possible in the time available to determine whether injection of insulin into isletectomised fish would restore the blood sugar to the normal level, but since it can diminish asphyxial hyperglycæmia it will probably be found to have this effect.

Summary and Conclusions.

1. The sugar in the blood of salt-water fishes immediately after catching varies considerably, both among individuals of the same species and among those of different species. Thus in *Myoxocephalus* (sculpin) it may vary from a trace to 35 mg. per cent.

2. The exposure of the fish to air, as in catching, causes marked hyperglycæmia, which sets in in about 30 to 45 minutes and may cause the blood sugar to rise to 160 mg. per cent. within one hour.

3. This hyperglycæmia can readily be induced by placing the fish in a limited volume of stagnant water, and in this its rate of development is accelerated by raising the temperature.

4. In two or three hours, under ordinary conditions of temperature, the sugar may rise to about 200 mg. per cent., but the extent to which it does so varies considerably in different individuals.

5. Replacement of the fish in frequently changing water, either in the sea or in a properly constructed aquarium, is not followed by return of the blood sugar to the normal level until after two to four days, or occasionally longer.

6. The amount of glycogen in the liver also varies very greatly in different individuals, but it is not possible, in most cases, to correlate this with the normal blood sugar or with the degree of the hyperglycæmia caused by asphyxia.

7. By hydrolysis of fresh blood with 0·1 N acid a marked increase in reducing power occurs, and a smaller increase may be detected when the protein-free filtrate is similarly hydrolysed. It is considered possible that a part of the asphyxial rise in blood sugar may depend on hydrolysis of non-reducing (masked) carbohydrates in the blood.

8. The average amount of fat in the liver of *Myoxocephalus* is 26 per cent.,

and it varies much less than that of glycogen. There is not usually a reciprocal relationship between fat and glycogen, so that the ratio of liver-weight to body-weight is no indicator of the amount of either, or both, of these reserve food-stuffs in the liver.

9. Glycolysis does not occur within 10 hours in the oxalated blood of *Myoxocephalus*, kept at room temperature, and taken from either normal or asphyxiated fish.

10. Intramuscular injection of epinephrin (adrenalin) causes marked hyperglycæmia, reaching its maximum in about two hours.

11. Intramuscular injection of insulin has only a slight effect on the blood sugar. This cannot be demonstrated on normal fish because the blood sugar is already at a low level and is often nearly absent. It can, however, be detected by subjecting fish previously injected with insulin to asphyxia, when the blood sugar rises less than would be expected without insulin. These effects of insulin have been obtained both after injecting the insulin just prior to inducing asphyxia and after injecting it daily for several days preceding the asphyxia.

12. Removal of the principal islets in *Myoxocephalus* is followed by marked hyperglycæmia. In isletectomised fish examined up to the fifth day after the operation, the blood sugar was found to be considerably above that of control fish that had been exposed to air for a period of time corresponding to that of the operation, or had been operated upon without actually removing the islets. After the fifth day the differences between the two groups of fish was much more striking, the controls usually showing blood sugars within the normal range, whereas in the isletectomised fish they were increased from three to twelve times the normal.

13. From the fifth to the eleventh days following isletectomy the hyperglycæmia became steadily less marked, but it was not possible to correlate this with the amount of glycogen in the liver.

14. There was more fat and less glycogen in the livers of isletectomised, as compared with normal fish.

We are indebted to Miss Marion Armour and Miss N. R. Hearn for making most of the analyses for glycogen and all of those for fat, and to the Biological Board of Canada and its director (Dr. A. G. Huntsman) for placing the excellent facilities of their Atlantic Station at our disposal. The expenses of the investigations have been partly defrayed by grants from the Carnegie Corporation.

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The Meiotic Phase in Triton (Molge vulgaris).

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(Communicated by Prof. J. B. Farmer, F.R.S.—Received June 19, 1924.)

[Plates 1–9.]

While it will be necessary to deal in greater detail at a later stage with some of the phenomena herein described, and with their bearing upon the work of others, I shall begin with a general description of my observations. I propose to adopt some of the terms used by Miss Digby in her paper on the "Meiotic Mitoses in Osmunda," in order to avoid confusion, and also because these observations on the Meiotic phase in Triton agree, at any rate as to some of the most important points, with hers on Osmunda.

"The term *thread* will be used to specify the longitudinal *half* of an entire univalent spireme or chromosome."

"The term *filament* will be used to specify the *entire* univalent spireme, the product of the close lateral association of two threads (*i.e.*, of two longitudinal halves of univalent spireme." I would add, or chromosome.