Studies on Fish in Japan as a Source of Insulin.

YUZO TOHYAMA, Sogo TETSUMOTO, Saburo FUKUYA
and Shuzo YAMADA

(Department of Food Control, The Government Institute for Infectious
Diseases, Tokyo Imperial University)

Insulin, the internal secretion of the pancreas, is formed in the islet tissue of that organ and passes from there into the general circulation. BRUNNER⁽¹⁾ (1682) suggested that the pancreas was in some way connected with the metabolism of fat and carbohydrates. Little progress was made, however, until the classic researches VON MEHRING and MINKOWSKI⁽²⁾ who discovered that the complete removal of the pancreas from dogs is followed by symptoms which closely resemble those observed in human diabetes mellitus. In 1846 STANNIUS⁽³⁾ described that the structures in the abdominal cavity of certain of the Teleostei. These were afterwards shown by DIAMARE⁽⁴⁾ (1899) to be homologues with the islets of Langerhans of the higher vertebrates. RENNIE⁽⁵⁾ (1903) examined the islets in 25 species of Teleostei, and usually found at least one encapsulated islets, which he called the "Principal Islet," of relatively large size in each species of fishes. Some 90 species of fish were subsequently examined by McCORMICK⁽⁶⁾ (1924) and also Japanese Fishes investigated by OHSAWA⁽⁷⁾ (1915), UKAI⁽⁸⁾ (1926), SUEHIRO⁽⁹⁾ (1941) and others. From these researches, we know that considerable variation was shown to exist among different species with regard to the position, size, and number of the islets.

BANTING and BEST⁽¹⁰⁾ succeeded in 1922 in preparing physiologically active extracts of the pancreas and clearly demonstrated the ability of these extracts to inhibit glycosuria and hyperglycemia in depancreatized animals and to alleviate the symptoms of diabetes mellitus in human beings. The insulin content of the islet tissue of the cod, the halibut and other common fish has been investigated by various workers such as MACLEOD⁽¹¹⁾, McCORMICK,⁽¹²⁾ VINCENT, DODDS and DICKENS⁽¹³⁾ and others. We also gathering the principal islets of tunny, bonito, cod, yellow-fish, mackerel and other fishes and extracted with picric acid and the insulin picrate was then extracted from the mass by moist acetone and converted to hydrochloride, mainly following to the DUDLEY's method⁽¹⁴⁾ (1924) which applied to the extraction of insulin from the islets of the cod and also another method. We used that the extraction of the fish islets with acid alcohol, namely the minced fish islets is extracted with 75 % ethyl alcohol containing 1.5 % of concentrated hydrochloric acid at 35°C and after adjusting pH, precipitated by acetone, washing with ace-

tone and ether, obtained white powder after drying in vacuo.

On standardization of insulin, the physiological method which is proved applicable for the determination of the activity of insulin than chemical method, we applied in our case the cross-over method of MARKS:⁽¹⁵⁾ taking six rabbits of approximately 2 kilograms, starved for 24 hours previous to the experiment, and divided into 2 groups of them and inject subcutaneously of suitable dose of the standard preparation of insulin into one half of a series of rabbits and the other half receiving simultaneously a dose of the sample of unknown unitage. Four to 7 days later the groups are crossed over and are used for the injection of the same preparations. Blood samples are usually taken at one and one half, at three, and at five hour interval after the injection or at every one hour within the course of five hours. We employed the HAGEDORN and JENSEN'S⁽¹⁶⁾ method for the determination of blood sugar. Thus we continued the experiment of the cross over method till the ratio of the blood sugar lowering between two series within 5 % and calculated the potency of our preparation. If we bring together the above experiments, they would be as Table 1:—

Table 1.

Name of Fish	Number of fish used	Yield of Lang erhans islet (g)	Yield of Ins ulin (g)	Percentage of yield (%)	International unit per mg. (1E)	International unit per g of L. islet. (1E)	International unit per Fish (1E)
Germo-germo (1)	809	80.00	0.5600	0.700	10.00	70.00	6.9
Germo-germo (2)	173	17.00	0.3400	2.000	Ca. 8.00	160.00	15.7
Germo-germo (Frozen)	610	60.00	0.6246	1.041	17.0	193.00	19.0
<i>Neothunnus macropterus</i>	97	13.00	0.2462	1.893	Ca. 9.10	172.34	22.9
<i>Parathunnus sibi</i>	114	15.0	0.2040	1.361	Ca. 10.00	136.10	17.9
<i>Katsuwonus vagans</i> (1)	665	60.00	0.3881	0.646	20.00	129.36	11.6
<i>Katsuwonus vagans</i> (2)	115	10.00	0.0545	0.545	Ca. 20.00	109.00	9.4
<i>Gadus macrocephalus</i>	310	25.00	0.2889	1.155	13.30	153.69	12.3
<i>Sebastes flammeus</i>	1070	72.90	0.9328	1.294	15.00	194.33	13.0
<i>Seriola quinqueradiata</i>	106	13.70	0.2172	1.585	Ca. 8.0	126.80	16.4
<i>Scomber Japonicus</i>	1370	20.60	0.1543	0.749	Ca. 13.00	97.30	1.5

If we calculate the value of fish in Japan as a source of insulin from our above experimental result, it would be found about 4,8389,0000 International Unit of insulin, as shown in Table 2, taking only 4 kinds of fish such as tunny, bonito, cod and yellow-fish, according to the government statistic 1938. It is difficult to know the exact number of consumption of insulin in Japan. However, if we assume to consume about 2,0000 International Unit per day: 730,0000 International Unit yearly, the above figure would be sufficient to supply about 66 years. From this calculation we can conclude that the source of insulin in Japan are quite inexhaustible.

Table 2.

Name of Fish	Yield of Fish 1936 (kwan)	Assumed weight per fish (kwan)	Yield of Fish (head)	Weight of Langerhans islet per head (g)	Total weight of Langerhans islet (g)	Weight of insulin as 1% extraction ratio (g)	Total International Unit 20 IE/mg (IE)
Tunny	900,9390	6	150,1565	0.10	15,0156	1501	3002,0000
Bonito	406,2218	1	406,2218	0.10	40,6222	4062	8124,0000
Cod	2898,9218	1	2898,9218	0.03	86,9676	8696	1,7396,0000
Yellow Fish	993,3536	2	496,6768	0.20	99,3355	9933	1,9867,0000
Total	5199,4362	—	3951,9769	—	241,9407	2,4192	4,8389,0000

Literature.

- (1) J. C. VON BRUNNER: *Experimenta nova circa pancreas*, 1682.
- (2) J. VON MEHRING and O. MINKOWSKI: *Arch. f. exper. path. u. Pharmacol.*, 26, 1890 371.
- (3) F. H. STANNIUS: *Anatomie der Wirbeltiere*. Berlin, 1846.
- (4) V. DIAMARE: *Internat. Monat. f. Anat. u. Physiol.*, 16, 1899 155.
- (5) J. RENNIE: *J. Anat. & Physiol.*, 37, 1903, 375.
- (6) N. A. McCORMICK: *Trans. Roy. Canad. Inst.*, 15, 1924, 57.
- (7) G. OHSAWA: *Nisshin-Igaku*: 5, 1915, 279.
- (8) T. UKAI: *Tohoku-Igaku-Zasshi*: 9, 1926, 37.
- (9) Y. SUEHIRO: *J. Imper. Fish. Experim. Stat.*, 11, 1941, 121.
- (10) F. G. BANTING and C. H. BEST: *J. Lab. & Clin. Med.*, 7, 1922, 464.
- (11) J. J. R. MACLEOD: *J. Metabolic Research*, 2, 1922, 149.
- (12) N. A. McCORMICK: *Bull. Biol. Board Canad.*, Dec. 1924.
- (13) S. VINCENT, E. C. DODDS & F. DICKENS: *Lancet*, 2, 1924, 115.
- (14) H. W. DUDLEY: *Biochem. J.*, 18, 1924, 665.
- (15) H. P. MARKS: *Brit. Med. J.*, 2, 1925, 102.
- (16) H. C. HAGEDORN u. B. N. JENSEN: *Biochem. Ztschr.* 135, 1923, 46.