to produce vaccine in large amounts the yolk sac technic is also easier to carry out and much less costly in time and materials.

Though the foregoing experiments were made with an eastern strain of spotted fever rickettsiae, we have also experimented with more virulent strains of the disease and with the Breinl strain of epidemic typhus rickettsiae. From our experience we are convinced that the conclusions just expressed are equally applicable to problems of making vaccine against the diseases caused by these other rickettsiae.

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Physiological and Toxicological Effects of Some Fish Muscle Extracts.

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While occasional descriptions of poisoning by fishes are encountered in ancient writings, surprisingly few scientific data of experimental nature on this subject are to be found in modern pharmacological literature Of medicinal agents derived from fishes and mentioned in modern materia medica probably the best known is cod liver oil, which physicians used empirically long before discovery of vitamin D. Another "fish" product employed in therapeutics is spermacetti, well known ointment base. With the advent of the vitamin era an intensive search was naturally made for these substances in other fishes than the cod, *e. g.*, in the halibut, haddock and menhaden. Another interesting study of what may be called a drug rather than a toxic agent was that made by Macht and Barba-Gosé on the oil expressed from the flesh and bones of the *Ruvettus pretiosus,* popularly known as the "castor-oil" fish.[1] This investigation was of special interest in respect to the chemical nature of the laxative principles contained in the oil.[2] Other poisons (not of putrefactive origin) are found in various fishes but few researches of an experimental nature have been published concerning them. Here belong the contributions made by Mosso[3] on the toxicity of the

[1] Macht and Barba-Gosé, *J. Am. Pharm. Assn.*, 1931, **20**, 558.

[2] Macht and Barba-Gosé, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 772.

[3] Mosso, *Arch. f. exp. Path.*, 1888, **25**, 111.

blood serum of certain *Muraena,* the studies by MacCrudden[4] on the choleriform effects produced by the ovaries of the barbel and the curious findings made by Japanese investigators with regard to the neurotoxic effect of extracts from the gonads of certain species of *Tetraodontidae* and *Diodontidae.*[5, 6] With the exception of that made on extracts from the *Ruvettus pretiosus,* no specific pharmacological research on the effects of fish *muscle* has been recorded. In connection with an extensive study, pharmacological and toxicological, on different animal poisons, and on snake venoms in particular, carried on by the senior author and his associates,[7] it was deemed interesting to inquire into the pharmacological properties of muscle extracts obtained from various fishes. Accordingly the writers began to study the biological effects on both animal and plant test objects of muscle extracts derived from all kinds of fishes.

Experiments have already been made in this laboratory with *freshly expressed* muscle juices of 65 different varieties, and new species of fish are continually being sought and subjected to careful study. In every experiment, the effects of decomposition were absolutely excluded. All the fish were placed in cold storage within a few hours of their capture and frozen solid and were kept in that condition until just before the experiment was begun. A piece of muscle for a preparation of the extract was then sawed out and the rest of the fish was replaced in cold storage. By "extracts" is meant the juice expressed from fresh muscle tissue without the use of any chemical solvent, the suspension in every experiment being prepared as follows:

Method of Preparation. Five to 10 g of fresh fish muscle are carefully minced and ground with an equal weight of clean sand and with 0.8% of physiological sodium chloride solution, 2 cc being employed for each gram of muscle. The emulsion or suspension produced by such painstaking grinding of the tissue is then strained or pressed through fine muslin and the filtrate is employed for pharmacological tests.

*Zoo*pharmacological experiments were made by injecting intraperitoneally 1 to 3 cc into white mice weighing from 20 to 25 g each. *Phyto*pharmacological experiments were performed by the methods introduced by Macht and his collaborators and described in various

[4] MacCrudden, *Arch. f. exp. Path.,* 1921, **91**, 46.

[5] Takahashi and Inoko, *Arch. f. exp. Path.,* 1890, **26**, 401.

[6] Ishiwara, *Arch. f. exp. Path.,* 1924, **103**, 209.

[7] Macht, *Trans. Am. Therap. Soc.,* 1940, **40**, 62.

papers published during the past 18 years.[8] This method, admirably suited to detection of presence of small quantities of animal poisons,[9] consists of studying the growth of *Lupinus albus* seedlings in plant-physiological solutions under standardized conditions described elsewhere. The increment or growth of the single straight roots of *Lupinus albus* seedlings is measured after the plants have been grown for 24 hours in the dark at 15°C or 60°F, and the growth of control seedlings in plant-physiological (Shive) solution is compared with the growth of similar seedlings from the same crop in 1% solutions of the muscle suspensions. The index of growth or phytotoxic index is expressed by the ratio of X/N or average growth in the unknown solution over the growth of the controls. The results obtained by means of the phytopharmacological test with 54 kinds of fresh muscle extracts are shown in Table I. The whole series of fish available was subjected to study under the standardized conditions described above. Most of the fish are of the edible type while others are not generally employed as food and still others are not eaten at all. It will be noted that some exerted a phytotoxic effect while others did not. Muscle suspensions made from all the non-poisonous and edible fishes gave indices of growth of not less than 70%. Most of the indices obtained were much higher, and some of the muscle extracts were actually stimulating for growth of the seedlings.

Nine species of fish, the names of which are underscored in the table, were definitely toxic for growth of *Lupinus albus* seedlings. The eel, formerly known to be poisonous only in respect to its blood serum, is included among the toxic fishes. It was also found that muscle extracts of *Spheroides maculatus,* or balloon fish or puffer, and of the porcupine fish were similarly toxic for plant growth. The saline muscle extracts of a tenth specimen, the bur-fish *(Chilomycterus),* were also found to be very toxic for the plants. In addition to these, muscle extracts of the catfish, skate, shark (dogfish and hammerhead), and *Opsanus tau,* or toadfish, exhibited phytotoxic reactions. Of special interest were the results obtained with the beautiful little *Vomer setipinnis,* popularly known as the moonfish. Several of this species, obtained in Atlantic City, were found to be markedly toxic for plants. Whether the so-called stingarees or sting rays are truly poisonous fish or injure human beings by the wounds which they make with their jagged spikes and which afterward become infected has long been a mooted question. Results of

[8] Macht, *Science,* 1930, **71**, 302.

[9] Macht, *Protoplasma,* 1937, **27**, 1.

TABLE I.
Toxicity of Fish Muscle Extracts.

No.	Common Name	Scientific Name	Phytotoxic Index, %
1	"Alewife"	*Pomolobus pseudoharengus*	82
2	Banded drum	*Larimus fasciatus*	80
3	Black bass	*Micropterus dolomieu*	80
4	Black drum	*Pogonias cromis*	105
5	Bluefish	*Pomatomus saltatrix*	80
6	"Bonito"	*Auxis thazard*	78
7	Bowfin	*Amiatus calva*	90
8	Butterfish	*Poronotus triacanthus*	81
9	Carp	*Cyprinus carpio*	90
10	Catfish	*Ameiurus catus*	48
11	Channel bass	*Sciaenops ocellatus*	80
12	"Chub"	*Chaenobryttus gulosus*	91
13	Cod	*Gadus callarias*	90
14	Croaker	*Micropogon undulatus*	90
15	Eel	*Anguilla chrisypa*	40
16	Flounder	*Paralichthys dentatus*	83
17	Flying fish	*Prionotus carolinus*	87
18	Gambusia		89
19	Goldfish	*Carassius auratus*	88
20	Haddock	*Melanogrommus aeglefinus*	80
21	Hake	*Urophycis regius*	98
22	Halibut	*Platysomatichthys hippoglossoides*	82
23	Herring	*Clupea harengus*	100
24	Kingfish	*Tarpon atlanticus*	83
25	Menhaden	*Brevoortia tyrannus*	90
26	Moonfish	*Vomer setipinnis*	51
27	"Mullet"	*Erimyzon sucetta*	87
28	Pike	*Esox americanus*	98
29	Pompano	*Trachinotus carolinus*	110
30	Porcupine fish	*Diodon hystrix*	60
31	Porgy	*Stenotomus chrysops*	80
32	Puffer	*Spheroides maculatus*	51
33	Rainbow trout	*Salmo irideus*	81
34	"Robin"	*Lepomis gibbosus*	91
35	Rock	*Roccus lineatus*	100
36	"Salmon trout"	*Cynoscion nebulosus*	96
37	Sand flounder	*Lophopsetta maculata*	85
38	Sand skate	*Pteroplatea maclura*	59
39	Smelt	*Menidia menidia*	90
40	Sea bass	*Centropristes striatus*	103
41	Shad	*Alosa sapidissima*	100
42	Shark (dogfish)	*Mustelus canis*	62
43	Silver squeteague	*Cynoscion nothus*	84
44	Spadefish	*Chaetodipterus faber*	80
45	Spanish mackerel	*Scomberomorus maculatus*	98
46	Spot	*Leiostomos xanthurus*	80
47	Stingaree	*Dasyatis say*	46
48	Sturgeon	*Acipenser oxyrhynchus*	87
49	Toadfish	*Opsanus tau*	49
50	Tuna, bluefin	*Thunnus thynnus*	88
51	Weakfish	*Cynoscion regalis*	97
52	White perch	*Pomoxis sparoides*	81
53	Whiting, Carolina	*Menticirrhus americanus*	84
54	Yellow perch	*Perca flavescens*	87

this series of phytopharmacological experiments support the view that in addition to such trauma stingarees produce a poisonous substance demonstrable in their muscle juices.

All the muscle extracts which proved to be poisonous for seedlings of *Lupinus albus* were profoundly toxic for white mice on intraperitoneal injection. Their toxicity was indicated by marked depression of all the animals followed by convulsions in some but in most cases by death due to paralysis of the respiratory center. Extracts of all the other fishes listed in the table, however, had no such toxic effect on mice. Four mice were usually employed in each animal experiment to examine those extracts which had been found to be poisonous for plants while in making the phytopharmacological test 2 or 3 sets of experiments were carried out with at least 10 seedlings for each solution studied.

Of great scientific interest in connection with this work were the findings made with extracts from various species of *Tetraodontidae* and *Diodontidae*, from certain of which some years ago Japanese scientists extracted the so-called "fugu" poison. Their descriptions of the poisonous effects of this substance indicate that it is a neurotoxin, *i. e.*, a chemical exerting its effect chiefly upon the central nervous system, and many of the symptoms it produces are not unlike those effected by bites of various serpents. The present writers having been engaged in an intensive pharmacological, toxicological, and therapeutic study of snake venoms and particularly of cobra neurotoxin have begun a chemical and pharmacological investigation of extracts of some of the *Tetraodontidae* and *Diodontidae* in order to compare their properties with those of snake venoms.

It is to be noted that all experiments were made with fresh unheated suspensions. Exposure to heat destroyed the toxicity of most of the muscle extracts with the exception of those from the *Tetraodontidae* and *Diodontidae*. These could be heated to 60°C without markedly impairing their toxicity for plants. Prolonged boiling of all the extracts (5 to 10 minutes) causes disappearance of the poisonous properties of all the solutions, a finding which is true of most poisons of animal origin, including snake venoms. The venom of the cobra has been found to be more resistant to heat than that of other serpents so that it could be repeatedly heated to 60°C without being completely inactivated.[10]

The phytopharmacological and zoopharmacological data described are of considerable historical interest. It will be noted that with

[10] Macht and Harden, *Am. J. Physiol.*, 1940, **129**, 411.

the exception of the catfish and eel, which are not ordinarily considered delicacies, all the "poisonous" fishes are inedible. Furthermore, all the fishes belonging to the toxic group underscored in the table possess either but rudimentary scales or none at all so that there seems to be some scientific basis for the ancient classification of edible and inedible fishes into two groups, namely, those which have scales and those which have not.[11]

Summary. (1) Extracts of muscle tissue prepared with physiological saline at room temperature were obtained from 65 varieties of fish and examined pharmacologically. (2) It was found that extracts of muscle tissue obtained from ten different sets of fishes were poisonous for seedlings of *Lupinus albus* and for mice while suspensions of the other fifty-five specimens exhibited little or no toxicity. (3) A comparative study of the chemical and pharmacological properties of the poisonous fish muscle extracts with those of certain snake venoms is now in progress.

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Effect of Repeated Administration of Delvinal Sodium [5-ethyl-5-(1-methyl-1-butenyl) barbituric acid] to Guinea Pigs.*

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Cope and Hancock[1] reported that the sodium salt of 5-ethyl-5-(1-methyl-1-butenyl) barbituric acid was an effective hypnotic with a high therapeutic ratio when administered either by stomach tube (oral) or by intraperitoneal injection. Hendrix[2] gave the same compound by stomach tube to both dogs and rats and stated that there was no indication of the development of tolerance to the drug in the case of dogs and that rats showed little or no tendency toward the development of tolerance.

In this paper, we wish to present the results of repeated intraperitoneal injections of delvinal sodium into 16 normal young guinea pigs.

11 Leviticus XI, 9-12.

* The delvinal sodium was kindly furnished by Sharp and Dohme, Inc., Philadelphia.

1 Cope, A. C., and Hancock, E. M., *J. Am. Chem. Soc.*, 1939, **61**, 776.

2 Hendrix, J. P., *J. Pharm. and Exp. Therap.*, 1940, **68**, 22.