by plasma "antithrombin," heparin serves merely as a catalyst. The amount of thrombin eventually destroyed by a given amount of plasma is dependent fundamentally upon the amount of co-factor present. The concentrations may be expressed by the following equation:

$$\log \frac{Y}{P} = 2.235 + 0.336 \log \frac{X}{P}$$

where Y is the amount of thrombin destroyed, X is the amount of thrombin remaining, and P is the amount of plasma (bovine) present in the reaction mixture.

14071

Reduction of Blood Pressure of Hypertensive Rats by Administration of Certain Marine Oils.*

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Our studies on the nature of the substance present in renal extracts which is effective in reducing the blood pressure in experimental hypertension have demonstrated that this active principle is of relatively small molecular weight.[1] This is evident from its chemical and physical properties as well as its effectiveness when administered orally. It was hoped, therefore, that sources other than mammalian kidney, in which the active principle is present in only small amounts, might be found as a more practical source of the material. Attempts to obtain active extracts from the kidney of fishes and amphibia have up to the present been unsuccessful. However, we have not been able to obtain large amounts of fresh tissue from these sources. Since these experiments have been in progress, there has appeared a recent report of Friedman, Soloway, Marrus and Oppenheimer,[2] in which certain quinones were demonstrated to have the capacity to reduce the blood pressure. We have been able to confirm these results. There has also appeared the report of the effectiveness of vitamin A,[3] which we have found to be relatively inert. It suggested itself, therefore, that the active material might be a derivative of the vitamin itself or one of the contaminants present in fish oils from which vitamin concentrates are prepared.

Methods. Rats have been used throughout in the present study. They were rendered hypertensive by fixation of the kidney by looping a stout cotton thread over the poles in the shape of a figure 8.† The blood pressures were determined by the plethysmographic method.[4]

The materials used in the present study consisted of pure provitamin A in the form of a mixture consisting of 90% β-carotene and 10% α-carotene and preparations and concentrates of various fish oils. These were administered orally by admixture with the animal's food. In addition to the preparations used in their natural form, some of the preparations were subjected to the following procedures which resulted in the destruction of vitamin A.

(1) Aeration by means of a current of air

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1 Grollman, A., Harrison, T. R., and Williams, J. R., *Fed. Proc.*, 1934, **1**, 34; in press.

2 Friedman, B., Soloway, S., Marrus, J., and Oppenheimer, B. S., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 195.

3 Pena, J. G., and Villaverde, M., *Rev. Cubana Cardiol.*, 1940, **2**, 322.

† This simplified procedure has proved to be superior to that in which silk is applied to the kidney, as originally suggested by Page.

4 Williams, J. R., Grollman, A., and Harrison, T. R., *J. Clin. Invest.*, 1939, **18**, 373.

TABLE I.

Effect of Various Vitamin A Preparations and Marine Oils on Blood Pressure of Hypertensive Rats. Each experiment was performed on a group of 5 animals; the values in table refer to amounts received by each animal and average drop in blood pressure observed.

Substances tested	Procedure to which test material was subjected	Quantity admin., g per rat	Original vit. A content U.S.P. units × 1000	Systolic blood pressure in mm mercury: Pre-treatment level	Reduction during week following beginning of treatment: Daily avg*	Max.†
β-carotene	—	0.2	200	180	0	2
Vit.-A concentrate	—	1.6	500	172	8	17
" "	Oxidation with H_2O_2	1.6	500	168	6	10
" " (alcohol)	—	0.4	400	191	5	10
" " "	Oxidation with CrO_3	0.4	400	170	4	8
" " (ester)	—	0.4	400	174	0	0
Cod liver oil	—	12.0	40	170	0	2
" " "	Aerated at 100°	12.0	40	162	0	3
" " "	Ultraviolet irradiation	12.0	40	169	0	5
" " "	Oxidation with CrO_3	6.0	20	172	12	14
" " "	" " "	5.0	8	186	16	24
" " "	" " H_2O_2	12.0	40	178	15	18
Crude fish oil	—	16.0	low	170	0	2
" " "	Oxidation with CrO_3	16.0	"	182	15	22
Sardine body oil (residue)	—	8.0	"	155	10	19
Sardine body oil (glyceride fraction)	—	8.0	"	168	0	4
Sardine body oil (fraction high in vit. A)	—	8.0	high	172	13	20
Sardine body oil (fraction low in vit. A)	—	8.0	low	180	12	20

*The values in this column were obtained by adding the differences between the pretreatment level and the observed levels on each day and dividing by the number of observations.

†The values represent the maximum average drop per rat observed on any day during the course of therapy.

for 10 hours, during which time the preparation was kept in a water bath at 100°C. (2) Irradiation with ultraviolet light from a mercury arc quartz lamp for one hour at a distance of 50 cm. (3) Oxidation with hydrogen peroxide (3% and 30%) in alkaline solution. The marine oil was stirred rapidly for 2 hours with an equal volume of 3% aqueous hydrogen peroxide, or with 10 cc of a 30% hydrogen peroxide solution with the addition of a drop of concentrated aqueous sodium hydroxide. The resulting emulsion was mixed with the animal's food and fed after drying in a current of air at room temperature. (4) Oxidation with CrO_3 in glacial acetic acid solution. To 20 cc of the marine oil was added 1 g of CrO_3 dissolved in the least possible amount of distilled water and 20 cc of glacial acetic acid. The mixture was stirred rapidly for 2 hours; made slightly alkaline with aqueous sodium hydroxide. After adding 100 cc of distilled water, the mixture was extracted repeatedly with ethyl ether and the resulting solution, after washing twice with a little water, was distilled *in vacuo* at 30°C. The remaining oil was mixed with the animal's food and administered in this form.

In addition to carotene, the following preparations have been studied: a concentrate of vitamin A in natural fish liver oil, containing 200,000 units per cc; a sample of esterified vitamin A, containing 500,000 units per g; and a sample of the free alcohol, containing a million units per g; codliver oil containing 1600 and 5000 units per g; a fish liver oil containing 60,000 units per g; ordinary commercial fish oil, dogfish oil, and sardine body oil. The last two named substances were tested in their original form and in fractions high in vitamin A, low in vitamin A, glyceride

fraction, and residues,‡ as prepared commercially by distillation.

The substances tested were administered orally in all cases by mixing them with the animal's usual food. The mixture thus obtained was readily consumed by the animals without wastage.

Results. In the accompanying table are reproduced such of the results as bear on the present problem. It is evident from these results that a variety of preparations derived from fish body or liver oils is capable of reducing the blood pressure of hypertensive rats. The results, however, indicate that the activity of different preparations is not proportional to their vitamin A content. In fact, oxidation which completely destroys all vitamin A activity enhances the effectiveness of the preparations in reducing the blood pressure. Destruction of vitamin A by heat and atmospheric oxygen does not increase this activity but more intensive oxidation by H_2O_2 or CrO_3 does so. Oxidation of vitamin A concentrates increases the activity little or not at all, the degree of activation depending rather upon the volume of oil used and its source, and not upon its original vitamin A content.

It would appear from the results of Table I that the agent responsible for the observed reduction in blood pressure is not derived from vitamin A but resides in some other constituent of fish oils which happens to be concentrated in commercial preparations of vitamin A. This would account for the observations of Pena and Villaverde[3] and of Wakerlin, Moss and Smith,[5] who have noted the capacity of vitamin A concentrates to reduce the blood pressure of patients and hypertensive dogs.

Refined fish oils, particularly those of marine origin, are more prone to auto-oxidation than the crude oils from which they are derived.[6] This would account for the presence of the material effective in reducing the blood pressure in the highly refined vitamin A concentrates, whereas, they are found in cruder preparations only after oxidation. On the other hand, the most highly purified vitamin

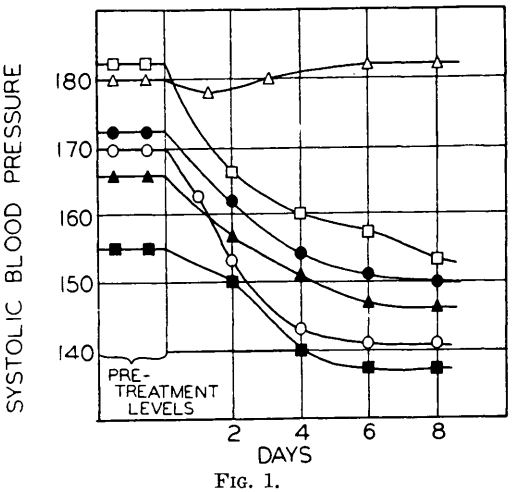

FIG. 1.

The effect of renal extract and various marine oils on the blood pressure of hypertensive rats.

Ordinates represent the systolic blood pressure in millimeters of mercury; abscissæ, time in days following a period of observation prior to starting treatment. All values represent averages obtained on groups of 5 rats, each series of which received the following admixed with their food on days 0 and 2 of the experimental period:

○ 20 g of renal extract derived from 50 kg pig's kidney.

● 22 g of cod liver oil containing 20,000 units of vitamin A after oxidation with CrO_3.

□ 40 g of crude fish oil oxidized with CrO_3

■ 40 g of a residual fraction of sardine body oil.

△ 40 g of cod liver oil containing 200,000 units of vitamin A after irradiation with ultraviolet light.

▲ 40 g of a vitamin A rich fraction of dogfish liver oil.

To conserve space the protracted return of the blood pressures to their pre-treatment levels is not indicated on the chart.

A concentrates, in which there is only a small amount of residual fish oil, are relatively inert.

Discussion. Until the respective substances are isolated, it is impossible to conclude about the relationship between the antipressor substances present in fish oils and kidney extracts. Preliminary studies demonstrate many simi-

‡ We are indebted to Mr. A. Lee Caldwell of the Eli Lilly and Company for the cod liver oil and vitamin A (alcohol and ester) concentrates; to Dr. L. M. Fox of the Winthrop Chemical Company for vitamin A in fish oil concentrate; to Dr. K. C. D. Hickman and the Distillation Products, Inc., for the dogfish and sardine oil fractions; and to Mr. W. V. B. Potter and the Beaufort Fisheries, Inc., for the crude fish oil.

5 Wakerlin, G. E., Moss, W. G., and Smith, E. L., *Science,* 1942, **96**, 161.

6 Buxton, L. O., *Ind. and Eng. Chem.,* 1942, **34**, 1486.

larities between the two as regards their general physical and chemical properties. They are almost identical insofar as their effect on the blood pressure is concerned, as shown in Fig. 1, which reproduces typical experiments not shown in Table I. Both are effective orally, manifest their action slowly and exert a relatively prolonged effect (a week or more) compared to the evanescent action which follows the depressor action of other substances.

The relatively high potency and ready availability of crude fish oils render these more satisfactory than kidneys as a source of the active principle effective in hypertension. Further experiments on the nature of the active substance are in progress. It is possible that a quinone analogous to those found to be effective by Friedman *et al.*[2] is formed by the oxidation of marine oils and that this is the agent responsible for the observed reduction in blood pressure.

Summary. Fish body and liver oils contain a substance which is effective in reducing the blood pressure of hypertensive rats. The effectiveness of these oils is increased by oxidative procedures which destroy vitamin A. The observed activity appears to be associated with some substance, other than vitamin A, present in fish oils. Fish oils offer greater promise than kidneys as a possible source of a therapeutic agent effective in reducing the blood pressure in hypertension.

14072

Precipitation and Concentration of Influenza Virus With Alum.*

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Influenza vaccines in which the virus has been concentrated by various methods produce a better antibody response than unconcentrated preparations.[1,2] Concentration of virus from the allantoic fluid of infected chick embryos has been accomplished by lyophilizing,[1] by adsorption on precipitates which result from thawing frozen extra-embryonic fluids,[2,3] by adsorption on and elution from chicken erythrocytes,[4] and, with mouse lung preparations, by adsorption on a precipitate of calcium phosphate and solution in sodium citrate.[5]

Alum has been used in the preparation of vaccines from bacteria and bacterial toxoids especially to increase the immunizing efficiency. This paper will describe a method for alum precipitation of the virus of influenza grown in the allantoic fluid of chick embryos.

Preparation of Virus Suspensions. Virus suspension diluted to 10^{-4} was inoculated into the allantoic cavity of 12-day-old chick embryos. The embryos were then incubated at 37°C for 2 days, candled, and the live eggs placed at 4°C overnight. Chilling the embryos facilitated collection of the allantoic fluid, and admixture of blood cells was avoided. The strain PR8 of type A influenza virus and the strain Lee of type B virus were used.

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† Associate Bacteriologist, United States Public Health Service, assigned to California State Department of Public Health.

1 Hirst, G. K., Rickard, E. R., Whitman, L., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1942, **75**, 495.

2 Hirst, G. K., Rickard, E. R., and Whitman, L., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 129.

3 Hare, R., McClelland, L., and Morgan, J., *Canad. Pub. Health J.*, 1942, **33**, 325.

4 Francis, T., Jr., and Salk, J. E., *Science*, 1942, **96**, 499.

5 Salk, J. E., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 709.