which has been considered to hinder formation of antibodies(6). Yet the immune responses in mongolians were not significantly different from those of the other children.

The finding that viremia was not observed in any of the 61 subjects should be emphasized in view of the recently advanced theory of Bodian that the absence of virus in the circulating blood precludes the development of paralysis(7).

Although the presence of virus in the stool could be demonstrated in 29 out of 61 patients, the antibody response would indicate that infection was established in many more cases, since the sera of only 6 patients had no neutralizing antibodies on the 30th day after ingestion of virus. One may possibly assume that in 5 out of these 6 patients the virus was destroyed before reaching the intestinal tract since no virus was isolated from their stool specimens. There was either insignificant or no rise in antibody response in 3 other patients whose blood did show some neutralizing power when drawn prior to feeding with virus. However, if one may draw a theoretical parallel with results obtained in cynomolgus monkeys(8), the level of antibodies observed in the latter 3 patients should be considered high enough to prevent paralysis after a peripheral exposure to virulent Lansing type virus. In any event, perhaps another oral administration of the TN strain to the 9 patients would result in multiplication of the virus in the intestinal tract and subsequent adequate serological response. On the other hand, a study of the genetic background of these 9 patients may throw additional light on their "non-reactivity" to the infectious process. The recent accumulation of data on the relationship between susceptibility to the paralytic form of poliomyelitis and the genetic constitution of the individual (9-11) may warrant investigation of the familial background of the 9 patients.

Summary. Sixty-one children who had no antibodies against Type II (Lansing) poliomyelitis virus were fed the TN strain of virus. None showed any clinical signs of illness. Viremia was absent in every case. In 29 individuals the virus was isolated from the stool from the 5th to the 20th day after feeding. Specific antibody rise was observed in most of the patients.

1. Koprowski, H., Jervis, G. A., and Norton, T. W., *Am. J. Hyg.*, 1952, v55, 108.

2. ———, Presented at 51st General Meeting, Soc. Am. Bacteriol., Chicago, May 1951. *Bact. Proc.*, 1951, 92 (Abs.).

3. Moyer, A. W., Accorti, C., and Cox, H. R., PROC. SOC. EXP. BIOL. AND MED., 1952, v —,—.

4. Neustaedter, M., *J. Am. Med. Assn.*, 1912, v59, 785.

5. Ludwig, Charles H., 1952, personal communication.

6. Siegel, M., *Am. J. Hyg.*, 1948, v48, 63.

7. Bodian, D., *Am. J. Hyg.*, 1952, v55, 414.

8. ———, *Am. J. Hyg.*, 1952, v56, 78.

9. Aycock, W. L., *Am. J. Pub. Health*, 1937, v27, 575.

10. Czickeli, H., *Schweiz. Med. Wochenschr.*, 1948, v78, 1092.

11. Herndon, C. N., and Jennings, R. G., *Am. J. Human Genetics*, 1951, v3, 17.

Received December 1, 1952. P.S.E.B.M., 1953, v82.

Heparin-Like Anticoagulants from Mollusca. (20092)

LAURENCE H. FROMMHAGEN, MARVIN J. FAHRENBACH, JOHN A. BROCKMAN, JR., AND E. L. R. STOKSTAD.

From Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

In the present investigation a study of a wide variety of aquatic life forms such as carp, whitefish, scallops, clams, and oysters revealed that 2 species of clams, *Mactra spissula* (sea surf clam) and *Artica islandica* (ocean quahog clam), contain unusually large amounts of heparin-like substances which exhibit high anticoagulant potency and low

TABLE I. Yield of Anticoagulant Activity in Crude Clam Extracts.

Species	Amt extracted, kg	Wt of crude extr., g	Anticoagulant activity of crude extr., U.S.P. units/mg	Anticoagulant activity/kg of meat, U.S.P. units × 100
Mactra spissula	82	310	10	374
	84	59	38	264
	91	747	6	484
Artica islandica	39	25	31	198
	86	864	4	396
	91	470	7	352

toxicity. We have designated the anticoagulant sulfated polysaccharides derived from *Mactra spissula* and *Artica islandica* as "mactin-A" and "mactin-B," respectively, the term being derived from the generic name of the sea surf clam.

This report deals with the extraction, purification, and certain biological, chemical, and physical characteristics of mactin-A and mactin-B.

Experimental. Isolation and purification. Crude extracts were prepared from the autolyzed meats of clams by alkaline extraction and subsequent proteolytic digestion. Representative activities and yields of the crude extracts are presented in Table I. All *in vitro* activities were determined by the U.S.P. sodium heparin assay(1) using sheep plasma. For comparison, the reported average yield of heparin activity from beef lung, one of the richest mammalian sources, was 18700 U.S.P. units per kg(2). An effective method of purification consisted of the following steps: The crude material obtained by precipitation with ethyl alcohol after proteolysis was dissolved in dilute alkali, then acidified to pH 1.5; protein impurities were denatured with gentle heating and removed by filtration; active material was precipitated in the filtrate by addition of ethyl alcohol, separated by centrifugation, redissolved in water, and finally isolated as a sodium, calcium or barium salt by addition of acetic acid. Purified products have been obtained with *in vitro* anticoagulant activities which range from 70 to 120 U.S.P. heparin units mg. Electrophoretic analyses (Fig. 1) of these purified samples demonstrated the presence of several components.

In vivo activity. The results of anticoagulant studies showed that both mactin-A and mactin-B possessed greater *in vivo* activity in New Zealand white rabbits than was demonstrated by the *in vitro* U.S.P. sodium heparin assay. The data in Fig. 2 represent average values obtained with mactin-B from 4 or more rabbits. Similar results were obtained by intravenous injection of equivalent doses in cats.

Toxicity studies. Purified samples of mactin-A and mactin-B with *in vitro* activities from 70 to 100 U.S.P. units/mg were used in all the following experiments. 1) The LD_{50}* in mice of a sodium salt of mactin-A was found to be 1.6 g/kg compared to 2.8 g/kg for standard U.S.P. sodium heparin. 2) *Acute toxicity.*† Single intravenous or subcutaneous injections of 10, 30, 50, and 100 mg/kg pro-

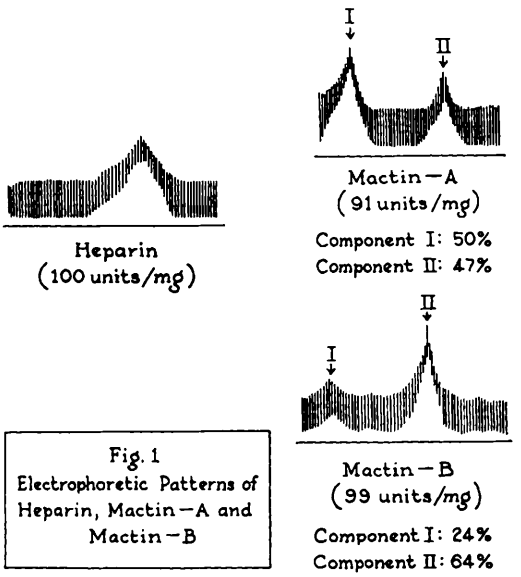

Fig. 1
Electrophoretic Patterns of Heparin, Mactin—A and Mactin—B

* Determined by Department of Pharmacology, Lederle Laboratories.

† Determined by Pharmaceutical and Chemical Testing Department, Lederle Laboratories.

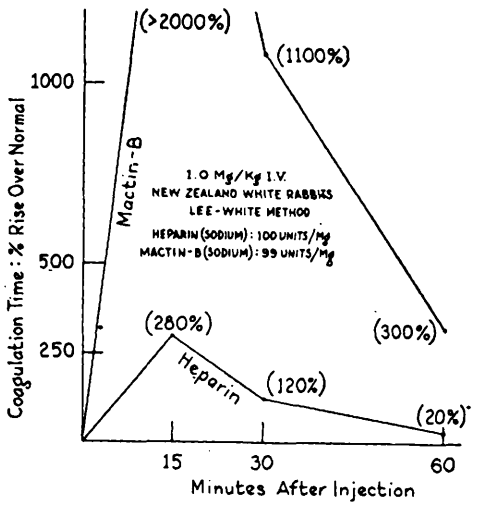

Fig. 2 In-Vivo Activity of Mactin-B and Heparin in Rabbits

duced no toxic effects in 2 New Zealand white and 2 Dutch belted rabbits. Two monkeys injected intravenously on 3 successive days with 2, 5, and 10 mg/kg exhibited no toxic reactions. No toxicity was observed in 50 mice and 40 rats after 5 daily subcutaneous injections of 10 mg/kg. 3) *Chronic toxicity.* Eight rats were injected intravenously with 20 mg/kg twice daily for 6 weeks with no evidence of hemorrhage or adverse effects on weight, activity, and appetite.

4) *Anaphylaxis.*† Three groups of 10 guinea pigs each were injected intraperitoneally with a sensitizing dose of 10 mg/kg. A challenging dose of 10 mg/kg administered 10 days later produced no anaphylactic symptoms. No antigenic effects have been noticed in any of the animals tested.

5) *Blood pressure.*† The blood pressure of 2 cats (histamine test) was not affected by intravenous doses of 5 and 10 mg/kg. 6) *Platelet count and agglutination.* A slight tendency of platelets to agglutinate in several clusters of 3 to 5 cells was first noted in New Zealand white rabbits at 10 mg/kg 15 minutes after injection; however, no decrease in platelet count was evident and agglutination was absent 30 minutes after injection. Neither platelet decrease nor agglutination occurs in the therapeutic range with highly purified samples. Further investigations of the effect on platelets of mactin-A and mactin-B at higher levels and under sustained conditions are in progress. 7) *Sedimentation.* No change of sedimentation rate has been noted in the blood of any of the animals tested.

Discussion. The crude product obtained after alkaline extraction and proteolytic digestion of both species of clams contained unexpectedly large quantities of anticoagulant activity determined by *in vitro* assay. In general, the yield of activity in crude clam extracts was found to be approximately twice the heparin activity obtained from a comparable weight of beef lung.

Since the electrophoretic patterns demonstrated the presence of at least 2 components in the purified samples of both mactin-A and mactin-B, it is obvious that the results of investigations concerning composition and structure may be subject to revision. However, a number of the tests used to determine the nature of heparin were applied to mactin-A and mactin-B. As with all sulfate esters of polysaccharides, a strong metachromatic color shift was obtained with both toluidine blue and azure-A. Carbohydrate color tests with mactin-A or mactin-B gave results similar to those obtained with heparin. Elemental chemical analyses have demonstrated a somewhat lower sulfur content for mactin-A and mactin-B than for heparin. Comparison of infra-red spectra of heparin, mactin-A, and mactin-B showed that the molecules were similar, but differences were noted in the positions of the absorption bands at wavelengths above 8 μ. The optical rotations of the sodium salts were almost identical with that of heparin. However, the intrinsic viscosities of both mactin-A and mactin-B were greater than that of heparin, which might be interpreted as evidence of higher molecular weight. In an effort to gain more information concerning the relative molecular weights of heparin and mactin-A and mactin-B, the technics of light scattering and the ultracentrifuge are being investigated.

Although a crystalline acid barium salt may readily be prepared from 100-unit heparin, all attempts to crystallize a corresponding acid barium salt of mactin-A and mactin-B with *in vitro* anticoagulant activities of approximately 100 U.S.P. units/mg have been un-

successful.

In vivo results in rabbits and cats revealed that sodium salt preparations of mactin-A and mactin-B with *in vitro* activities of approximately 100 units/mg were more active than preparations of standard 100-unit sodium heparinate. The observed intensity of action was more than 6 times that of heparin; the duration of action was also significantly greater. A satisfactory explanation for this wide discrepancy between the *in vitro* and *in vivo* activities has not been found.

As in the case of heparin the anticoagulant effect of mactin-A is neutralized by injection of protamine.

While the LD_{50} of mactin-A of 1.6 g/kg is lower than the value of 2.8 g/kg established for heparin. the anticoagulant activity of mactin-A is greater than that of heparin; therefore, the therapeutic index of mactin-A would appear to be comparable to that of heparin. Acute and chronic toxicity studies conducted thus far have revealed no apparent toxicity. Clinical trials of mactin-A and mactin-B are in progress.

Summary. Heparin-like polysaccharides with high anticoagulant activity have been isolated from 2 species of clams, *Mactra spissula* and *Artica islandica*. These purified polysaccharides, termed mactin-A and mactin-B, are related to but not identical with mammalian heparin. They possess a greater *in vivo* activity and a favorable therapeutic index when compared with heparin preparations of equal *in vitro* activity.

We wish to thank Dr. Raymond Brown for the electrophoretic analyses, and Mr. L. Brancone and associates for microchemical analyses.

1. Pharmacopeia of the United States, 1950, v11, 271.

2. Kuizenga, M. H., and Spalding, L. B., *J. Biol. Chem.*, 1943, v148, 641.

Received December 19, 1952. P.S.E.B.M., 1953, v82.

Liver Regeneration: Preliminary Report.* (20093)

CHARLES G. CHILD, III, DAVID BARR, GEORGE R. HOLSWADE, AND CHARLES S. HARRISON.

From the Department of Surgical Research, Department of Surgery, New York Hospital and Cornell University Medical College, New York City.

The reason for the failure of hepatic regeneration following partial hepatectomy in dogs with Eck fistulae is not clear. Some relate this to diversion of a factor peculiar to portal blood and others simply to reduction in total blood flow through the liver. In an attempt to discover whether portal blood is essential to hepatic regeneration an operation was devised in dogs that accomplished complete diversion of the portal stream and at the same time provided the liver with a profuse supply of systemic venous blood. In this preparation the response of the liver to partial hepatectomy was studied.

Method. In one group of dogs the portal vein and inferior vena cava were divided below the liver and continuity re-established by end to end anastomosis crossing portal to systemic and caval to portal circulations. (Fig. 1). One month later 70% hepatectomy was performed. Hepatic venograms, obtained by injecting contrast material directly into the inferior vena cava, were made prior to hepatectomy to prove patency of the caval-hepatic anastomosis and to visualize the intrahepatic portal bed. Venography was repeated at intervals afterward to demonstrate regeneration *in vivo*. After periods of observation ranging from 35 to 60 days post-hepatectomy the dogs were sacrificed by exsanguination under nembutal anesthesia. Their livers were removed and weighed and the degree of regeneration calculated. The assumption was made, based upon dissections of normal livers, that a 70% hepatectomy

* Supported by U. S. Public Health Service Grants-in-Aid.