Inhibition of Lipemia Clearing Activity by Tissue Extracts.* (24142)

EDMUND KLEIN, WALTER F. LEVER, AND LASZLO L. FEKETE
(Introduced by Chester N. Frazier)
(With assistance of Dewey M. Duckett, Jr. and Walter E. Grueninger)
Department of Dermatology, Harvard Medical School, Mass. General Hospital, Boston

The serum of patients with primary or secondary hyperlipemia inhibits heparin-induced lipemia clearing activity(1). After high-speed centrifugation of hyperlipemic serum, inhibitory activity is located in the supernatant fatty layer(2). Seifter and Baeder(3) showed that a dialyzable inhibitor of lipemia clearing is present in the blood of rats. Hollett and Meng(4) separated a fraction from normal human serum which inhibited heparin-induced lipemia clearing activity. The studies presented here were undertaken in order to determine whether inhibitors of the lipemia clearing system are present in the tissues as well as in the blood. Such studies seemed to be further indicated by the presence of clearing activity in the tissues(5).

Materials and methods. Aliquots of a number of tissues were obtained from human autopsies† and from exsanguinated rabbits. *Tissue extracts* were prepared by differential centrifugation after repeated washing of tissue aliquots in 0.85% NaCl by a modification of the method of Schneider(6). Washed specimens were homogenized in one volume of saline at 5°C and centrifuged at 2500 g for 30 minutes at 4°C. The sediment was resuspended in one volume of saline and kept at −15°C for 24 hours.‡ After thawing the suspension was centrifuged as before. The supernatant was passed through a Seitz filter and further cleared of particulates by centrifugation at 32,000 g for 30 minutes at 4°C. (Saline Extract). *Solubility characteristics* of the inhibitory tissue components were determined in pH range between 2 and 12. In the acid pH range 0.1 M sodium citrate-citric acid buffers, and in the alkaline range 0.2 M sodium glycinate-glycine buffers were used. Extraction was carried out in the respective buffers as described above for saline. Extracts were dialyzed at 15°C against running tap water for 24 hours and distilled water for 1 hour, and then centrifuged at 32,000 g for 30 min. at 4°C. The supernatants (Glycine Extracts and Citrate Extracts, respectively) were kept at 5°C until used. *Effects of extracts* on heparin-induced clearing activity were determined by optical density measurements, glycerol evolution and electrophoretic mobility of lipoproteins. For these determinations 0.6 cc of extract was mixed with 0.6 cc of human post-heparin§ or fasting serum. To each mixture 0.1 cc of solution containing 100 N.I.H. units of thrombin‖/cc of physiological saline were added. Mixtures were incubated at 37°C for 10 min. and clots removed after fluid had been expressed from them. Then, 0.3 cc of standard fat emulsion¶ was added and the final mixture was incubated at 37°C. The degree of clearing was determined spectrophotometrically according to method of Grossman(7). Electrophoretic mobilities of serum alpha and beta lipoproteins in this mixture were determined by means of paper electrophoresis as previously described(8). Glycerol determinations were carried out on aliquots of mixtures prior to and after 1 hour incubation at 37°C with Korn's modification

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‡ Higher levels of inhibitory activity resulted from freezing and thawing the tissues.

§ Post-heparin serum prepared as previously reported(1).

‖ Thrombin (Topical Thrombin, Parke, Davis & Co., Detroit, Mich.) was added to remove residual fibrinogen which otherwise formed fibrin during optical density measurements and thus interfered with these determinations.

¶ The standard fat emulsion contained 15% coconut oil, 0.5% Pluronic (non-ionic detergent) and 1% polyglycerol oleate, supplied by Upjohn Co., Kalamazoo, Mich.

TABLE I. Inhibition of Heparin-Induced Lipemia Clearing Activity by Saline Extracts of Human Tissues.

Tissue	No. of specimens	Avg % of inhibition	Stand. dev.
Spleen	22	93	7
Kidney	24	90	7
Liver	21	89	8
Psoas muscle	7	78	8
Lung	6	68	11
Testis	5	62	19
Adrenals	8	47	12
Heart	14	38	20
Thyroid	3	30	8
Aorta	6	28	9
Adipose tissue	7	24	2
Skin	12	21	2
Brain	5	21	1
NaCl (control)		0	

(9) of the method of Lambert and Neish(10).

Results. Biochemical studies. Saline extracts of human and rabbit tissues inhibited lipemia clearing in post-heparin serum determined by optical density changes, glycerol evolution and electrophoretic mobilities of serum alpha and beta lipoproteins. There were differences, however, in inhibition by various tissues. Optical density determinations (Table I) revealed inhibition in extracts of all human tissues examined. Levels of inhibition in rabbit tissues corresponded with those in analogous human tissues. Variations in inhibition in any rabbit tissue were considerably less than in corresponding human tissue and did not exceed 8%. Liberation of glycerol from triglycerides in post-heparin serum was inhibited completely by saline extracts of tissues with strongly inhibitory activities (*i.e.*, kidney, spleen and liver). The method was not sufficiently sensitive for evaluating extracts which were moderately inhibitory by optical density measurements.

Increase in electrophoretic mobilities of alpha and beta lipoproteins, which reflects clearing activity in a mixture of fat emulsion with post-heparin serum, was inhibited by saline extracts of kidney, spleen and liver. Such inhibition did not occur, however, with saline extracts of other tissues. Sediments removed by high-speed centrifugation of kidney, spleen and liver extracts, when resuspended in saline, had between 10 and 30% of the inhibitory action of the first saline extracts. There was slight or no inhibitory activity in sediments obtained from other tissues.

Physical chemical studies. Dialysis of saline extracts of spleen, kidney, liver and heart against water or physiological saline resulted in 30 to 40% loss of inhibitory activity. Exposure of these extracts to increasing temperatures (15°-100°C) for 30 min. led to increasing loss of inhibitory activities. At 100°C the loss was 80 to 100%. Decreasing pH to 4.2 resulted in reversible precipitation of inhibitory components. Extraction with glycine buffer of human and rabbit kidney, spleen and liver was optimal at pH 7.5 to 8.0. In this pH range glycine extracts had between 80 and 100% of the inhibitory activities of corresponding saline extracts. Incubation of glycine extracts of kidney and liver with trypsin at 37°C for 12 hours resulted in 70 to 100% loss of inhibitory activities.

Control studies. Fasting serum was used in place of post-heparin serum in optical density determinations. No changes occurred, indicating that tissue extracts did not interfere with spectrophotometric determinations. Addition of heparin** either to tissues (kidney, spleen and liver) prior to extraction, or to extracts, did not effect their inhibitory activities. Use of Protamine in place of tissue extracts, in concentrations of 10μ/g/cc of saline did not inhibit clearing as determined by measurements of optical density and glycerol evolution.

Discussion. Our data indicate that extracts of a number of human and rabbit tissues inhibit heparin-induced clearing activity of serum, although degree of inhibition varied in different organs. Inhibition was least in tissues which normally have a high fat content (adipose tissue, brain) or which represent sites of pathological deposition of lipids (skin, aorta).

Loss of inhibitory activities of extracts which results from dialysis appears to be due to a dialyzable inhibitor. Since the inhibitory effects of the nondialyzable materials are

** Heparin sodium (Upjohn Co., Kalamazoo, Mich.) was added, 0.5 to 10 μg/g of tissue or /cc of tissue extract.

reduced by trypsin or heat, some of the inhibitory components appear to be proteins.

Failure of heparin to reduce inhibitory activities of tissue extracts furthermore suggests that inhibition is not the result of simple deactivation of clearing factor as a consequence of complex formation with polycations. Thus, *in vitro* inhibition of tissue clearing factor by Protamine which, as reported by Korn (8). is reversible by *in vitro* addition of heparin, differs from inhibition exerted by extracts described here.

Summary. Extracts of human rabbit tissues inhibited heparin-induced lipemia clearing activity of serum. Extracts of spleen, kidney and liver were strongly inhibitory; those of other organs exercised slight or moderate inhibition. The tissues contained inhibitory components which appear to be associated with proteins since they are nondialyzable. heat-labile and inactivated by trypsin.

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Bioassay of Ataractics Against Lethal Action of Mescaline in Mice. (24143)

N. P. PLOTNIKOFF AND H. WASHINGTON (Introduced by C. A. Stone)
Merck Institute for Therapeutic Research, West Point, Pa.

Current interest in mescaline has stimulated a search for agents with antagonistic actions. Thus, Deegan and Cook(1) have reported the protective effects of various centrally active agents against pruritic episodes induced by mescaline in mice. Spech(2) reported that mescaline induced bradycardia and hypoglycemia in rats, which could be reduced with fasting and epinephrine. The same author reported that mortality was unaffected by the use of barbiturates, curare, decamethonium or physostigmine.

It is the purpose of this study to demonstrate the protective effects of various ataractic agents against mescaline-induced mortality in mice.

Methods. Toxicity of mescaline in female CF_1 mice (18-22 g) was determined using the intravenous route. The LD_{95} was found to be 150 mg/kg in 610 animals. The typical response consisted of a very brief clonic seizure terminated by respiratory arrest in a matter of 2 to 3 minutes. The agents under study were administered intraperitoneally one hour prior to treatment with mescaline, with the exception of the following: meprobamate (20 min), benactyzine (5 min), diphenylhydantoin (40 min), reserpine (24 hours), trimethadione (40 min), serotonin (30 min), morphine (30 min), and amphetamine (30 min). Only base weight of the drug was used in determining dosage. Ten mice were used per dose level and at least 3 doses per drug were examined. In this study the end point of measurement was the survival or death of the animal 24 hours following mescaline injection. The per cent protection was plotted on log-probit paper and the effective dose 50%, the slope, and the fiducial limits were calculated according to the methods of Litchfield and Wilcoxon(3).

Results. In Table I are shown the PD_{50}'s (protective dose 50%) and slopes of the protective agents with their respective fiducial