Inhibitory Effect of Human Blood Components on a Lipase of Microbiological Origin.* (26282)

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Investigation of lipid metabolism in patients with hyperlypemia raised the possibility of presence of lipase inhibitors in plasma. Tauber(1) reported the presence of antilipase activity in human, rabbit and guinea pig serum and in laked rabbit red blood cells against the lipolytic activity found in rabbit serum. In the present study, it was found that normal human plasma and red blood cells inhibited lipolytic activity of a lipase of microbiological origin. An attempt was made to determine in what fractions of blood this inhibitory activity resided.

Materials and methods. Olive oil was used as substrate and a mixture of 3 ml of a stable olive oil emulsion and 0.5 ml of a phosphate buffer at pH 6.56, as described by Tauber (1), was used for each experiment. The results in Table I were obtained using one mg of a microbiological lipase (Lipase B, Mix 4A, Rohm and Haas Co.)† and incubating at 37°C with shaking for 20 hr, and in Tables II and III using 10 mg of a microbiological lipase (Mann Research Laboratories, Inc.)† and incubating at 37°C with shaking for 1.5 hr. Following incubation, 2 drops of phenolphthalein and 3 ml of 95% ethanol were added, and the mixture was titrated with NaOH.

Freshly drawn blood from normal nonfasting adults was used. The red blood cell hemolysate was made by adding 5 ml red blood cells to 6 ml water and the red blood cell ghosts by hemolyzing 5 ml red blood cells in water, washing the centrifugate 4 times with water, and resuspending in 10 ml water. The hemoglobin solution contained 0.15 g per ml water. The red blood cell dialysate was made by adding 12 ml red blood cells to 12 ml water in a cellophane dialysis bag which was allowed to stand in 30 ml water at 4°C for 19 hr. After removing dialysate, the bag was dialyzed in running water for 30 min. The plasma dialysate was made similarly, using 3 ml plasma against 40 ml water. The ashed solutions were remade to original volume.

Results. Both plasma and a red blood cell

TABLE I. Effect of Human Blood Components on Lipase Activity.

Sample	Vol, ml	Before* (ml NaOH)	After* (ml NaOH)
RBC hemolysate	.5	.94	4.30
" "	.1	.96	—
" ghosts	.5	7.58	7.69
Hemoglobin	.5	1.35	3.64
RBC-d.	2.0	4.30	6.80
" heated‡	2.0	5.08	6.71
" ashed	2.0	5.33	6.68
RBC-n.	2.0	1.16	5.84
" heated‡	2.0	1.94	4.72
" ashed	2.0	6.75	6.58
WBC-1§	.5	6.26	6.85
" 2§	.5	5.58	5.57
Plasma	1.0	1.44	7.93
" no enzyme	1.0	1.25	—
Plasma-d.	2.0	5.96	7.76
Plasma-n.	.5	2.00	8.27
" heated‡	1.0	4.85	8.16
Serum-heated‡–supernatant	.5	5.48	7.17
" precipitate	.5	1.99	7.19
Water	2.0	5.16	6.80
" no enzyme	2.0	1.00	—
Enzyme control†	—	7.54	1.47
No enzyme control‖	—	1.15	—

* Titration values in ml 0.046 N NaOH for addition of sample before and after incubation.

† Titration values are for addition of enzyme before and after incubation of the emulsion and buffer mixture.

‡ Heated at 100°C for 8 min.

§ WBC-1 prepared by Method #2 of Walford *et al.*(2) (WBC from 40 ml blood suspended in 7 ml isotonic saline); WBC-2 prepared by method of Lapin *et al.*(3) (WBC from 35 ml blood suspended in 7 ml isotonic saline).

‖ Contained emulsion and buffer only.

Abbreviations are as follows: RBC—red blood cells; d.—dialysate; n.—non-dialyzable fraction; WBC—white blood cells.

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† Prepared from a species of fungus in the Aspergillus oryzae-niger group.

TABLE II. Effect of Human Plasma Protein Fractions on Lipase Activity.

Fraction	Before* (ml NaOH)	After* (ml NaOH)
I	3.88	7.97
II	4.60	7.25
III-0	4.50	7.35
III	4.22	7.57
IV-1	4.40	7.78
IV-4	3.78	7.35
V	4.07	7.79
Enzyme control†	8.10	2.08
No enzyme control‡	1.80	—

Fifty mg of each fraction was added in 0.5 ml water. Fractions and descriptions thereof were generously supplied by Dr. R. B. Pennell, Protein Foundation, Inc., Jamaica Plain, Mass. Composition of these fractions is as follows: I—fibrinogen, antihemophilic globulin, cold insoluble globulin, etc.; II—gamma globulin; III-0—β-lipoprotein; III—clotting components, isoagglutinins, plasminogen, etc.; IV-1—α-lipoprotein, ceruloplasmin; IV-4—β-metal combining protein, serum esterase, α-lipoprotein, albumin, etc.; V—albumin. Fractions I, IV-1, IV-4, and V were prepared by the method of Cohn *et al.*(4); fractions II, III-0, and III were prepared by the method of Oncley *et al.*(5), using as starting material fractions II + III obtained by the former method.

* Titration values in ml .047 N NaOH for addition of fraction before and after incubation.

† Titration values for addition of enzyme before and after incubation of the emulsion and buffer mixture.

‡ Contained emulsion and buffer only.

hemolysate inhibited enzyme activity, whereas white blood cells did not (Table I). White blood cells showed no appreciable lipolytic activity when incubated with the emulsion and buffer only, even after sonication and addition of taurocholate; thus, an inhibitory effect did not appear to be masked by any lipolytic activity of the white blood cells. Red blood cell ghosts appeared to have no effect on enzyme activity, but a hemoglobin preparation showed inhibitory activity. The non-dialyzable portion of a red blood cell hemolysate showed inhibitory activity, which was destroyed by ashing but not by heating at 100°C for 8 min; the dialysate appeared to have some inhibitory activity, which was destroyed by heating at 100°C for 8 min and by ashing. The non-dialyzable fraction of plasma showed inhibitory activity which appeared to be partly destroyed by heating at 100°C for 8 min, but the dialyzable fraction appeared to be inactive. Likewise, the precipitate obtained by heating serum at 100°C for 8 min was inhibitory, but the supernatant apparently was not. No inhibitory activity was seen with urine or with heparin, which was used as anticoagulant. From Table II, it can be seen that human plasma protein fractions obtained by cold ethanol fractionation all showed some inhibitory activity. The inhibitory effect of human serum albumin appeared to be a function of its concentration (Table III). The microbiological lipase (Mann) also hydrolyzed triacetin but not Tween 20. Albumin, however, did not inhibit triacetin hydrolysis. Pork pancreatic lipase (Nutritional Biochemicals Corp.) was found to hydrolyze the olive oil emulsion, but was not inhibited by plasma, red blood cells, or albumin. Albumin, likewise, did not inhibit triacetin and Tween 20 hydrolysis by this enzyme. Wheat germ lipase (Mann) did not hydrolyze the olive oil emulsion but did hydrolyze triacetin.

The activity of both microbiological lipases showed little change when initial pH of the incubating mixture was varied over the range of 6.4 to 7.0. Addition of the protein fractions, plasma, or red blood cells did not move the pH of the mixture out of this range before incubation. Moreover, in the samples tested (red blood cells, fraction V, enzyme

TABLE III. Effect of Human Serum Albumin on Lipase Activity.

Albumin, mg	Before* (ml NaOH)	After* (ml NaOH)
1.6	7.05	7.33
3.2	5.39	—
6.3	4.60	—
12.5	4.20	7.47
25.0	4.02	—
50.0	3.50	—
100.0	3.57	7.50
Saline—1 ml	7.19	6.97
Enzyme control†	7.72	1.50
No enzyme control‡	1.15	—

Each quantity of the lyophilized human serum albumin (Cutter Laboratories) used was added in 1 ml isotonic saline. Dialyzing the albumin solution did not appreciably alter the results.

* Titration values in ml 0.047 N NaOH for addition of fraction before and after incubation.

† Titration values for addition of enzyme before and after incubation of emulsion and buffer mixture.

‡ Contained emulsion and buffer only.

control), the pH did not change more than 0.1 unit as a result of incubation. This pH stability may have resulted from the insolubility of the fatty acids produced allowing little or no effect on pH. The inhibition of enzyme activity by the materials tested, therefore, could not be accounted for by an effect on pH of the incubating mixture.

Discussion. It appeared, therefore, that protein components most likely accounted for the lipase inhibition seen with plasma and red blood cells. Tauber(1) in fact, reported presence of serum antilipase which inhibited lipolytic activity normally found in some sera; he also found that laked rabbit red blood cells inhibited serum lipase activity and concluded that hemoglobin was the inhibitor. The ineffectiveness of ashed plasma and red blood cells in the present study indicated that inorganic electrolytes were not responsible for the inhibition.

The mechanism of an inhibitory activity of plasma proteins and hemoglobin on this enzyme is unknown. If the enzyme were autocatalytic, as reported for a lecithinase (6), the protein could prevent activation of the enzyme by binding the fatty acid products. The lack of albumin inhibition on triacetin hydrolysis by this enzyme, however, is evidence against this possibility, and suggests that the inhibitory effect of albumin and the other protein fractions on enzyme hydrolysis of the olive oil emulsion might have been the result of coating of the lipid droplets by these proteins, thereby preventing access of the substrate to the enzyme.

Summary. Human plasma, red blood cells, plasma protein fractions and a hemoglobin preparation were found to inhibit lipolytic activity of a microbiological lipase preparation when an olive oil emulsion was used as substrate. Human serum albumin, which was inhibitory in proportion to its concentration, was not inhibitory when triacetin was used as substrate. Hydrolysis of the olive oil emulsion by pork pancreatic extract was not inhibited by human plasma, red blood cells or albumin.

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Influence of Age on Liver Phospholipide Metabolism of Mice.* (26283)

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Phospholipides are involved in many basic structures of the cell, constituting 16-32% dry weight of nuclei, mitochondria and microsomes(1), and are chemically part of certain enzymes(2). To our knowledge, the influence of age upon radioactive turnover and chromatographic separation of the individual phospholipides has not been previously reported.

Methods. Alteration of phospholipide metabolism and distribution with increasing age was studied in inbred strains of female mice (AxZb). Six hours before sacrifice 25 μc of $NaH_2P^{32}O_4$ was administered intraperitoneally. This time interval corresponds to the ascending part of the specific activity

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