On the contrary similar antiserum treatment gave very poor protection to the virus in sensitized mice. Apparently the state of hypersensitivity impaired the protecting qualities of the antibodies.

The observations presented in this report may have special interest for prophylactic work. Due to the present-day mass immunization of children with various bacterial antigens (including *H. pertussis*) and the possible increase of the number of cases of bacterial allergy, the regular test for the safety of a new vaccine on normal animals may not be adequate. Our studies offer a very simple technic for testing a new vaccine on hypersensitive mice.

Summary. Experiments with mice indicate that hypersensitivity induced by the *H. pertussis* antigen can increase susceptibility to influenza virus.

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New Olive Oil Emulsion for Lipase and New Observations Concerning "Serum Lipase". (22038)

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Emulsions prepared from olive oil, or similar fats, tend to separate on standing after short periods of time. For tests of lipase activity stable emulsions are required. In addition, there is in these tests a correlation between the degree of emulsification and the amount of lipase activity detected(1). In the present paper a simple method for preparing a stable emulsion of olive oil is described. With this emulsion the "lipase activity" of various sera was determined. In addition the following points were investigated: (a) the activation of serum lipase by Lima bean trypsin inhibitor, (b) a method for demonstrating and determining antilipase in serum, (c) other observations on lipase.

Methods. Preparation of a stable olive oil emulsion. The emulsion is a conventional olive oil-acacia mixture to which there is added 0.25% of a new hydrophilic colloid "Carbopol 934" (B. F. Goodrich Chemical Co.). Carbopol 934, having carboxyl groups, disperses in water to yield a low viscosity acid solution which on neutralization turns into a stable gel. Toluene (0.5%) is also incorporated into the emulsion to prevent bacterial growth. Control tests demonstrated that toluene does not inhibit lipase action. One gram Carbopol 934 and 200 ml of distilled water were mixed in Waring blendor until dispersion was complete (5 minutes). A solution of 5 g of acacia (McKesson, U.S.P.) in 100 ml of distilled water was added to the Carbopol solution; the mixture was stirred in a Waring blendor for 5 minutes and then filtered through glass wool. The blendor must be thoroughly washed at this time to remove the small particles of Carbopol which would interfere because Carbopol is a strong acid. The colloid mixture was then returned to the blendor, and 100 ml of olive oil (Fisher, U.S.P.) were added in a slow stream during 5 minutes with stirring. Without discontinuing mixing, N sodium hydroxide was then added dropwise until a pH of 7.00 was obtained. Usually 10.3 ml N sodium hydroxide

were required. Finally, 2 ml of toluene were added dropwise and homogenization was continued for 15 minutes. Air from the emulsion was removed in a desiccator with the aid of a pump. The emulsion may be kept in a refrigerator at 4° for several weeks without separating and without an appreciable increase in its titration blank. Brief shaking of the emulsion, however, is advisable before it is used. Digests prepared from this emulsion did not separate during the digestion period. When Carbopol was omitted from the emulsion the olive oil separated within a few hours. Cotton seed oil, as marketed in this country, cannot be used as a lipase substrate since it contains natural lipase inhibitors. *Phosphate Buffer.* 5.54 g of monobasic potassium phosphate and 3.41 g of anhydrous dibasic sodium phosphate were dissolved in distilled water and made up to 1000 ml. A few drops of toluene were added to prevent bacterial growth. The pH of buffer was 6.56. *Enzyme Preparations.* Human plasma cholinesterase was prepared from Cohn fraction IV-6 by Cutter Laboratories. It possessed 800-times the cholinesterase activity of whole plasma. Wheat germ lipase was obtained from Worthington Biochemical Corp.; the pancreatic lipase from Nutritional Biochemical Corp. Normal human sera were obtained from nonfasting blood donors (North Carolina Memorial Hospital); the other sera from nonfasting animals. *Crude Lima bean trypsin inhibitor (LBI).* This globulin fraction of Lima beans was prepared according to Tauber, Kershaw and Wright(2).

The lipase test. 1-ml portions of serum or enzyme solution were placed in 30 x 125 mm Pyrex test tubes. To each tube 5 ml of olive oil emulsion and 0.5 ml of phosphate buffer were added. The tubes were stoppered and shaken for 1 minute and then placed in a constant temperature water bath for 20 hours at 37°. Controls were prepared by adding the serum or enzyme solution to the olive oil emulsion-buffer mixture at the end of the digestion period. The control values were subtracted from the total values. At the end of 20 hours 3 ml of 95% ethyl alcohol and 2 drops of a 1% solution of phenolphthalein in ethyl alcohol were added. After mixing, the digests were placed in 30-ml beakers and titrated with 0.05 N sodium hydroxide. A 2-ml Koch micro burette was used to which soda lime tubes were attached to prevent absorption of CO_2 by the alkali. The mixtures were stirred with a glass rod after the addition of each drop of alkali. The solutions were titrated to a deep pink color, and the pH's (9.70-9.80) were checked with a Beckman Model G pH meter. All values are expressed in ml 0.05 N sodium hydroxide. *Kinetics of serum lipase.* Hydrolysis of the olive oil within 48 hours was fairly linear with respect to time. The following titration values (minus controls) were obtained: 24 hours, 1.55; 48 hours, 2.90; 72 hours, 3.92. The lipase activity was not proportional to increasing quantities of rabbit serum. 0.1 ml serum gave a value of 0.74; 0.2 ml gave a value of 1:00; and 1.0 ml gave a value of 1.65.

Keeping quality of serum lipase. Serum lipase appears to be a fairly stable enzyme. Different sera, when kept for 24 hours at 37°, lost none of their lipase activity when compared to specimens kept in deep freeze at −18°, and at 4° for the same length of time.

Serum antilipase. The presence of antilipase in inactive serum may be demonstrated by adding some of the inactive serum to some of the active serum. For instance, when 0.2 ml of inactive guinea pig serum was added to 1 ml of active rabbit serum the titration value dropped from 1.41 to 0.45 ml. In sera that have lipase activity the antilipase may be demonstrated by keeping an aliquot of the serum for 30 minutes at 56°. This destroys all but a trace of the lipase but not the antilipase. In the antilipase test, for convenience and because of its fairly high lipase content, dilute rabbit serum may be employed as the lipase source. The rabbit serum is diluted about 10 times in order to give a titration value of 1.2 ml with 0.05 N sodium hydroxide, after 20 hours. 0.4 ml of heated human serum is added to the dilute rabbit serum. The serum mixtures are made to 1.6 ml with 0.85% sodium chloride. After 30 minutes of interaction, the lipase test is applied. Inhibition of dilute rabbit serum by different quantities of "inactivated serum," however, is not quite proportional to the quantity of inacti-

vated serum employed. This is probably due to the presence of a trace of active lipase in the heated serum. *Inhibition of serum lipase by the antilipase of the same serum* may be demonstrated by adding 0.4 ml of heated rabbit serum (30 minutes at 56°) to 1 ml of 10-times diluted rabbit serum. Here the inactivated heated serum, containing the antilipase, brought about almost complete inhibition of the unheated serum lipase. The olive oil test was done in the usual manner. *Hemoglobin, a lipase inhibitor.* Hemoglobin is also a powerful inhibitor of serum lipase. Saline-washed rabbit red cells were frozen, thawed, laked with 20 volumes of distilled water and cleared by centrifuging. The lipase activity of 1 ml of rabbit serum was lowered from 1.54 to 0.73 when 0.2 ml of the laked rabbit red cell solution was added. For this reason sera obtained from hemolyzed blood cannot be employed in lipase assays.* *Lipase activity of different biological materials obtained with olive oil emulsion.* The following lipase values were obtained: human serum, 0.00-0.84 (average 0.22); rabbit serum, 0.50-1.70 (average 1.37); guinea pig serum, 0.00-0.25 (average 0.27); rat serum, none. Sixteen samples of human sera and 10 each of the other species, were employed. For other enzymes the following titration values were obtained: pancreatic lipase, 1.05; human plasma cholinesterase, 0.20; wheat germ lipase, 0.60. The digests contained 1 ml serum or 1 ml (20 mg) of enzyme solution, and 0.5 ml phosphate buffer. The digestions were carried out in a constant temperature water bath for 20 hours at 37°, except the pancreatic lipase-containing digest which was kept for 30 minutes in a constant temperature water bath. Only the water soluble portion (the filtrate) of the pancreatic lipase was used. The final pH of the serum digests was 7.00; the final pH of the other digests was 6.3.

Activation of serum lipase by crude Lima bean inhibitor (LBI). Table I shows typical experiments concerning the activation of serum lipase by the Lima bean inhibitor. This globulin fraction of Lima beans is a potent inhibitor of crystalline trypsin, a growth inhibitor of mammals(2), and an inhibitor of fibrinolysin(3). It was of interest to find out how lipase activity would be affected by LBI. It is seen in Table I that LBI is a powerful activator of human, rabbit, horse, and rat serum lipase. Boiling the LBI destroys some of its activity. LBI itself had no lipase activity. The activating action on serum lipase by the LBI cannot be explained at this time. Isolated serum proteins had no activating effect.

TABLE I. Activation of Serum Lipase by LBI.

Type of serum	LBI, mg	Titration values, ml — Total	Minus control	Increase
Rabbit*	0	2.50	1.40	
"	1	3.00	1.90	.50
"	4	3.52	2.42	1.02
"	5	3.50	2.40	1.00
"	10	3.50	2.40	1.00
"	5†	2.77	1.67	.27
None	20	1.16	.06	
"	0	1.10 (control)		
Human*	0	1.58	.48	
"	5	2.35	1.25	.77
Horse (fasted)	0	1.20	.10	
"	5	1.95	.85	.75
Rat (fasted)‡	0	1.12	.02	
"	5	1.70	.60	.58

* Pool of 6 different specimens.
† Boiled 10 min.
‡ Pool of 18 specimens.
The digests contained 1 ml of serum. The LBI was dissolved in saline solution. The digests without LBI contained saline solution instead.

Summary. A stable olive oil emulsion has been described. Using this new emulsion the lipase activity of a series of biological materials have been studied. Sera of different mammals showed different lipase activities. Sera of some species had a wide range of activity, whereas, those of others had no activity. Wheat germ "lipase" splits olive oil only slightly. Highly purified plasma cholinesterase was almost inert indicating that this enzyme is not involved in fat digestion. Hemoglobin is a powerful inhibitor, and Lima bean inhibitor, a powerful activator of serum lipase. A method for demonstrating antilipase in serum has been presented. It is well known that normal human serum and the

* We are indebted to Dr. Paulo Seabra for calling this fact to our attention.

serum of most other mammals do not contain a specific lipase similar to pancreatic lipase. The hydrolysis of olein described in this paper is affected by another type of enzyme. For convenience the term lipase has been employed in this paper.

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Variation in Response of Various Mouse Strains to Hexobarbital (Evipal). (22039)

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The use of inbred strains of mice, as an established experimental procedure in areas of medical research other than cancer, has been less extensive for various reasons, particularly because of the lack of information concerning characteristics of these established strains other than tumor type and incidences. Various investigators have determined some of the other characteristics these strains exhibit. Russell, Newfeld, and Higgins(1) have investigated the normal blood picture in 18 inbred mouse strains; Law, Morrow, and Greenspan (2) have investigated the liver glucuronidase activity in inbred strains; Tuba(3) has determined serum tributyrinase levels in 3 inbred strains; and Wragg and Spiers(4) have demonstrated inbred strain differences in response to adrenal cortical hormones.

The data herein reported is additional information on strain differences in mice, and is concerned with the response to a barbiturate drug, hexobarbital (Evipal), as measured by sleeping time.

Materials and methods. The various strains tested, shown in Table I, came from the production colonies of the National Institutes of Health. Initially, the investigation was concerned only with closely inbred strains, but because of the existence of some doubt about the uniformity of inbred strains in research of this sort(5), it was decided to extend the observations to additional strains in order to test other genetic situations. The first 10 strains listed in the table have been closely inbred by brother x sister matings, so they represent a genetic condition in which maximum homozygosity is to be expected. The CFW strain has been mated brother x sister in a large closed colony with no effort made to maintain close relationships between matings, so that many family lines are maintained parallel in each animal generation. Genetically, such a population will no doubt exhibit considerable homozygosity, but is expected to be more variable than the more closely inbred strains. The non-inbred Swiss strain has been random

TABLE I. Mean Sleeping Time in Minutes for Various Strains of Mice Injected with 125 mg per Kilo Body Weight of Evipal. All animals were males, 70-80 days old.*

Strains	No. of animals	Mean sleeping time	Stand. dev.
A/LN	25	48.44	4.17
DBA/2JN	64	46.83	4.22
A/HeN	25	41.25	1.92
BALB/cAnN	63	41.01	1.99
C57L/HeN	29	32.52	3.22
C57BL/6JN	37	32.26	5.71
C57BR/cdJN	25	31.93	5.51
C3HfB/HeN	30	22.02	2.99
BRSUNT/N	26	18.34	3.34
SWR/HeN	38	18.08	4.15
CFW	32	46.43	11.75
Swiss (non-inbred)	47	43.39	14.51

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