diabetic control group. In the absence of testosterone propionate therapy diabetic animals did not produce significantly greater amounts of fructose than did castrated control animals with normal blood sugar levels. It appears that the male sex hormone is in some manner necessary for *in vivo* formation of seminal fructose from blood glucose, even when the latter substrate is present in excessive amounts. These data do not elucidate the mechanism whereby testosterone controls this conversion.

1. Mann, T., Biochem. J., 1945, v39, 458.
2. ———, ibid., 1946, v40, 481.
3. Mann, T., Parsons, U., Nature, Lond., 1947, v160, 294.
4. Lutwak-Mann, C., Mann, T., Price, D., Proc. Roy. Soc. B, 1949, v136, 461.
5. Mann, T., Parsons, U., Biochem. J., 1950, v46, 400.
6. Mann, T., Lutwak-Mann, C., ibid., 1948, v43, 266.
7. ———, ibid., 1951, v48, 16.
8. ———, Physiol. Rev., 1951, v31, 27.
9. Hers, H. G., Biochim. Biophys. Acta, 1960, v37, 120.
10. ———, ibid., 1960, v37, 127.
11. Samuels, L.T., Harding, B. W., Mann, T., Biochem. J., 1962, v84, 39.
12. Roe, J. R., J. Biol. Chem., 1934, v107, 15.
13. Linder, H. R., Mann, T, J. Endocrinol., 1960, v21, 341.

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Lung Lipase Levels in Normal Rats and Rats With Experimentally Produced Fat Embolism.* (31896)

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Fat embolism is a condition in which fat appears in the circulating blood, not in the fine emulsion of a metabolic lipemia, but in droplets large enough to occlude arterioles and capillaries. Recent experiments on rats in our laboratory indicate that the lung is the only organ or tissue which captures fat droplets in any significant quantity when it is injected intravenously as triolein(1). Embolic fat disappears from the lung of the rat over a period of a few days after injection(1). While some is removed by phagocytosis, it appears that the major portion is mobilized by enzymatic breakdown of the neutral fat. That the lung secretes lipase as one of its many nonrespiratory functions has been known for some time(2,3).

This experiment was designed to measure changes of lung lipase activity in rats following the injection of a measured amount of triolein. The heart, which is also known to have lipase activity(3), was used as a control organ.

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Methods. Twenty-four pairs of Holtzman male rats from 6 to 11 weeks old were used. Each pair consisted of a rat injected with 0.25 ml triolein and a rat of the same age and approximate weight injected with 0.25 ml of 0.9% saline solution. Both rats were carried together through the experimental procedure of injection, sacrifice, preparation of acetone powders, and lipase determinations.

Forty-eight hours after injection each rat was given 0.15 ml of sodium pentobarbital (65 mg/ml), the abdomen was opened and the animal was sacrificed by withdrawal of blood from the aorta. The heart and lungs were removed and placed in cold 0.9% saline.

Acetone powders of each organ were prepared, weighed and stored in a desiccator at —18°C until lipase determinations were performed.

Extracts of acetone powders were obtained for lipase assay, by mincing one-half of the acetone powder from each organ with 8 ml distilled water, shaking, incubating for 15 minutes at 37-38°C, shaking again and centrifuging. The aqueous layer was removed

TABLE I. Lipase Assay Values in ml 0.05 *N* NaOH/ml Extract or Serum.

No. of rat pair	Serum			Heart			Lung		
	Triolein injected	Control	Injected minus control	Triolein injected	Control	Injected minus control	Triolein injected	Control	Injected minus control
1	1.39	1.18	.21	.48	.67	—.19	1.05	.93	.12
2	1.31	1.17	.14	.59	.57	.02	.78	.98	—.20
3	1.27	.91	.36	.10	.10	.00	.40	.18	.22
4	1.11	1.12	—.01	.14	.10	.04	2.49	.25	2.24
5	1.17	1.44	—.27	1.04	.75	.29	2.47	2.30	.17
6	1.84	1.30	.54	.59	.91	—.32	2.95	1.32	1.63
7	1.36	1.10	.26	.47	.42	.05	1.13	.66	.47
8	1.55	1.13	.42	.10	.15	—.05	1.26	.15	1.11
9	1.24	1.47	—.23	.31	.18	.13	.93	.54	.39
10	2.67	1.19	1.48	.40	.43	—.03	2.98	.86	2.12
11	1.89	1.19	.70	.39	.43	—.04	1.14	.86	.28
12	.99	†		.18	.23	—.05	.71	.31	.40
13	.44	.90	—.46	.22	.15	.07	.48	.24	.24
14	1.32	.59	.73	.27	.53	—.26	.69	.70	—.01
15	.96	2.21	—1.25	.48	.40	.08	1.32	1.03	.29
16	.03	.24	—.21	.54	.54	.00	.90	.72	.18
17	.28	.20	.08	.55	.58	—.03	2.29	1.30	.99
18	—.06	*		.39	.38	.01	1.77	1.40	.37
19	.27	.21	.06	.04	.11	—.07	1.14	.45	.69
20	1.67	1.43	.24	.44	.38	.06	1.66	.70	.96
21	1.22	1.19	.03	.02	.02	.00	.36	.37	—.01
22	.68	†		.28	.31	—.03	2.01	.60	1.41
23	1.32	1.02	.30	.16	.15	.01	.73	.27	.46
24	.82	1.08	—.26	.19	.18	.01	1.95	.93	1.02
Mean			.14			—.01			.65
Standard deviation			.52			.12			.67

* This serum sample was accidentally dropped.
† Sera obtained was insufficient for the lipase test, due to blood-clogged syringe.

with a disposable pipette and recentrifuged. This procedure was repeated until a clear extract was obtained.

The tissue lipase method was adapted from the Comfort and Osterberg modification of the Cherry and Crandall method for serum lipase assay(4) as follows. Calcium-treated olive oil: Heat 40 ml olive oil† and 0.9 g $CaCO_3$, with constant stirring, to 100°C. Filter through a Buchner funnel until clear. Substrate: Mix together 280 ml distilled water, 120 ml phosphate buffer,‡ 40 ml calcium-treated olive oil and 8 ml acacia (gum arabic, powdered) in a Waring Blendor for 15 minutes. This emulsion is stable for two weeks when stored in the refrigerator. Procedure: Ten ml of the emulsion are incubated for 24 hours at 37-38°C with 1 ml tissue extract or 1 ml serum. Ten ml absolute ethanol are added to the incubation flask to inhibit further lipase activity, and the incubation mixture is then titrated with 0.05 *N* NaOH to a pale pink phenolphthalein endpoint. Blanks were run to determine the titratable acidity of the substrate and the acidity of extract or serum prior to incubation. Serum blanks consisted of 10 ml substrate incubated for 24 hours at 37-38°C, and mixed with 1 ml refrigerated serum at the time of ethanol addition, just prior to titration. In the case of tissue extract determinations, blanks run in this manner were not significantly different from the values obtained with substrate incubated alone. To simplify the procedure, blanks consisting of 10 ml substrate without extract were incubated for 24 hours at 37-38°C, 10 ml ethanol added and the mixture titrated in the same manner as the test mixture. Four blanks were run with each set of determinations, and the average of these values taken as the blank for all of the tissue extract determinations run at that time. The value for the blank was sub-

†O-111 olive oil obtained from Fisher Scientific Co.
‡M/15 phosphate buffer solution, pH 7.0 prepared by Hartman-Leddon Co. for Cherry and Crandall serum lipase determination. Harleco #23667 obtained from Matheson Scientific Inc.

tracted from the value of each test titration to give the assay value due to lipase activity.

Results. Dissection of rats 48 hours after injection with triolein revealed lungs with hemorrhages and increased weight over the lung weight of the paired control. No hemorrhages were noted in the lungs of any of the control animals. The hearts of triolein injected rats did not differ from those of the paired controls either in appearance or in weight.

Table I gives the lipase assay values in ml of 0.05 *N* NaOH per ml extract or serum. One ml extract represents 1/16 of the activity of the heart or lung. Lipase tests on the sera indicated slightly higher activity in the injected rats; however, the difference was not statistically significant. Lipase tests on the heart showed no significant difference between the triolein injected and the paired control rats. Lung lipase values of the triolein injected rats, however, were significantly greater than those of the paired controls, using the Wilcoxon matched-pairs, signed-ranks test, $\alpha = 0.005$(5). It will be noted that one control serum (Pair #15) and one control lung (Pair #5) are unusually high. The reason for this is unknown. Both of these saline-injected rats had lungs which were clear of hemorrhage and within the control weight range; therefore, they were included in the series.

Summary. The gross and histological changes in the lungs of rats injected with triolein are similar to those seen in the lungs of patients dying with fat embolism. An elevation of the serum lipase activity in patients with fractures is diagnostic of pulmonary fat embolism(6). The result of this experiment indicates that there is an increase in the amount of lipase activity in the lung in experimental fat embolsim in rats, and emphasizes the role of enzymatic breakdown in the removal of embolic fat from the lung. It also suggests that the source of the excess lipase in the serum may be the lung.

1. Paredes, S., Comer, F., Rubin, S., Adler, F., Peltier, L. F., J. Bone Joint Surg., 1965, v47-A, 1216.
2. Gomori, G., Arch. Path., 1946, v41, 121.
3. Korn, E. D., J. Biol. Chem., 1955, v215, 1.
4. Comfort, M. W., Osterberg, A. E., J. Lab. Clin. Med., 1934, v20, 271.
5. Siegel, S., Nonperametric Statistics, 1956, p19, 83.
6. Adler, F., Peltier, L. F., Clin. Orthopaedics, 1961, v21, 226.

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Trace Proteins in Biological Fluids. IV. Physicochemical Properties and Sites of Formation of γ-Trace and β-Trace Proteins.* (31897)

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Two proteins of beta and gamma mobility were initially described as occurring in higher concentration in human cerebrospinal fluid (CSF) than in serum(1). Subsequently, these globulins were further characterized by the effect of enzymes and storage upon their electrophoretic mobilities(2), and behavior on column chromatography(3). The concentrations of β-trace and γ-trace were determined in the CSF of patients with and without neurological disease. It was concluded from these studies that the γ-trace protein represented approximately 2% and the β-trace 10% of

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