or activator of plasma protease. It may be recalled that crystalline pancreatic trypsinogen is activated to trypsin by a mold kinase.[10]

Addendum. In a recent note (*Nature*, 1948, **161**, 559), published after our original communication[4] and received while the present article was in press, C. H. Lack reports confirmation of the staphylo*kinase* factor.

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Oleic Acid Toxicity and Fat Embolism.*

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In view of the paucity of data on the toxicity of fatty acids, experiments with oleic acid were performed. In control tests, olive oil was injected intravenously, and observations were made pertaining to fat embolism.

The hemolytic effects of fatty acids are a well known phenomenon. This report deals with toxic manifestations observed in addition to those of hemolysis. The intravenous injection of fat emulsion was studied by a number of investigators such as Murlin and Riche;[1] Kochne and Mundel;[2] Gordon and Levine;[3] Clark and Brunschwig;[4] Dunham and Brunschwig;[5] McKibbin *et al.*;[6] Shafiroff and Frank;[7] LeVeen;[8] and reportedly by Russian workers who used human fat.

Johnson *et al.*[9-11] have demonstrated increased erythrocyte fragility after the ingestion of large fat meals, due to fatty acids and soaps which enter the blood with the lymph of the thoracic duct.

Dubois *et al.*[12] and Davis[13] demonstrated that serum albumin has considerable affinity for oleic acid, thereby inhibiting the bacteriostatic effects and toxicity of the latter.

For assays of toxicity, 36 normal, starved, unanesthetized, mongrel dogs were used. A few assays were performed on dogs anesthetized with pentobarbital sodium, in which carotid blood pressures were recorded.

The substances used were oleic acid (95% oleic acid and 5% other long chain fatty acids), ethyl, oleate, and sodium oleate; the latter 2 were prepared from the oleic acid. All substances were administered intravenously. The oleic acid and the ethyl oleate and valuable advice were obtained by the kindness of Dr. A. W. Ralston of the Armour Laboratories.

Results. Doses of 0.25 cc of oleic acid had little effect (6 dogs), while doses of 0.5 to 5 cc were toxic (19 dogs). The outstanding

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symptoms were: drop in blood pressure, urination and defecation, and rapid, forced, deep respirations, coughing, and development of massive pulmonary edema with large amounts of bloody, frothy exudate from the mouth and nose, followed by collapse and death. Life after injections of 5 cc lasted from 5 minutes to 2 hours, with an average of 40 minutes.

Hemolysis occurred, varying in degree from 75 mg % of free hemoglobin to almost complete hemolysis (13.54 gm per 100 ml), depending on the dose of oleic acid given. In animals with low degrees of hemolysis, the hematocrit was elevated. Blood pH usually dropped to the acid side, from 7.46 to 6.37; the lowest value of 6.37 was obtained in a moribund animal, that died within a few minutes. If an animal survived for 1 hour or more, blood pH returned to normal or to alkaline values; the highest alkaline pH observed was 8.2. Serum calcium dropped to 6-7 mg % in most animals, and in 2 dogs to as low as 2 and 0.6 mg %; the latter values appeared in moribund animals that showed the highest degrees of hemolysis.

Autopsy showed the following: the gastrointestinal tract was markedly contracted, especially the pyloric antrum of the stomach; however, the cardia and fundus of the stomach were dilated. The urinary bladder was contracted maximally. The most significant changes were found in the chest. The thoracic cavities contained much bloody fluid. the lungs were edematous and hemorrhagic throughout, and on section bloody fluid oozed from the parenchyma and from the bronchi. The amount of hemorrhage and edema far exceeded any condition which might be ascribed to fat embolism.

Microscopically, in formalin fixed frozen sections, stained with scarlet red and hematoxylin eosin, red masses in the pulmonary vessels varying in size from specks to globules, massive edema of the lungs, and intestinal hemorrhages were observed. Heart, liver, kidneys, and adrenals, were found to contain fat staining material, however not to a strikingly larger extent than tissues from normal controls; that is lipids in the hepatic cells, in the bile duct cells and in the heart did not appear to be increased or altered by the injection of oleic acid.

Three dogs were injected repeatedly with sublethal doses of oleic acid. They presented the symptoms of increased and forced respiration as described above, but recovered within a day. They seemed to acquire a tolerance to oleic acid which manifested itself by decreasing hemolysis and, after 12 injections, practically no hemolysis was observed. The livers in these chronic dogs were pale yellow and friable, and showed microscopic evidence of fatty metamorphosis.

In order to analyze whether the toxic effect of oleic acid was due to the free carboxyl radical, ethyl oleate was injected in doses of 0.5 to 5.0 cc, (3 dogs). Ethyl oleate was toxic, showing the same clinical symptoms as oleic acid but no edema or hemorrhage of the lungs and no hemolysis, and it was never lethal. Animals sacrificed 1-2 hours after injection did not show the pulmonary changes seen with oleic acid, either grossly or microscopically.

Oleic acid forms soaps in an alkaline medium. In order to analyze the possibility that the toxic effects of oleic acid were due to this, sodium oleate (2 g) was injected (3 dogs). The animals died within a few minutes, showing nearly complete hemolysis.

Comparable amounts of pure olive oil (5 cc) injected intravenously gave no untoward reactions nor hemolysis (2 dogs). Microscopic sections revealed large amounts of fat-staining material in the capillaries of the lungs, much more than with any of the other materials studied, namely oleic acid, ethyl oleate and sodium oleate; yet olive oil was the least toxic of all substances tested. The dogs acted normally and did not seem to be affected in the least. The massiveness of fat embolism as seen microscopically after olive oil injection and the lack of symptoms do not seem to fit too well into the clinical picture of fat embolism.

Conclusions and summary. If, according to the findings of Davis and Dubois, albumin has the power of binding oleic acid in the ratio of 9 to 1, then the amounts of oleic acid in-

jected exceeded the protective range in the dogs used in our experiments.

The mechanism of the toxic action of oleic acid is problematical. Small doses of sodium oleate produced a degree of hemolysis never seen with comparable doses of oleic acid.

While changes in blood serum calcium appeared to be correlated with the degree of hemolysis, the low values of serum calcium may be explained by formation of calcium soaps. Changes of blood pH appeared to be associated with respiratory conditions and with acid and toxic effects of the oleic acid.

Oleic acid does not seem to produce massive edema of the lungs by embolism, but by toxic effects on the capillaries of the lungs. Caution must be used in the preparation of fat emulsions for intravenous alimentation, in order to exclude fatty acids and soaps. Fat embolism *per se* may not be so dangerous unless fatty acids are released in or into the circulation.

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In vitro Effect of Streptomycin and Para-Aminosalicylic Acid (PAS) on the Growth of Tubercle Bacilli.*

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On the basis of Bernheim's[1] observations that the sodium salts of benzoic and salicylic acids specifically stimulated the oxygen uptake of the tubercle bacillus, Lehman[2,3,4] undertook to find a substance of similar chemical structure which might prove bacteriostatic for this organism. Of the 60 derivatives of benzoic acid studied, 4 amino-2-hydroxy benzoic acid (para-aminosalicylic acid or PAS) was found to be the most effective, inhibiting growth of the tubercle bacillus *in vitro* in concentrations as low as 0.15 mg %. This effect was found to be bacteriostatic rather than bacteriocidal. Sievers[5] and Youmans[6] soon confirmed these findings. Youmans reported that 6 streptomycin-resistant strains of tubercle bacilli tested by him were equally sensitive to PAS.

Smith and McClosky[7] and others have reported that a combination of two bacteriostatic agents often is more effective against the tubercle bacillus than either drug alone. It was logical to assume, therefore, that PAS plus streptomycin might behave in a similar manner; in this paper we report the *in vitro* effects on the tubercle bacillus of each of the 2 drugs alone and in combination.

While these experiments were in progress, Youmans[8] reported that the *in vitro* bacteriostatic effect of PAS in combination with streptomycin was no greater than the sum of the individual activities of the two substances.

Methods. Cultures were grown in the liquid medium described by Dubos,[9] and the concentration of tubercle bacilli in mg/cc was determined by turbidimetric readings in calibrated tubes using a blue filter (420 mμ) in a Lumetron colorimeter.

The following 2 test organisms were used: (A) the human strain of tubercle bacilli H-37RV, which is streptomycin-sensitive and (B) the naturally occurring strep-

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