of great importance in the therapy of meningitis, and it is suggested that intermittent intravenous injection of penicillin may most promptly establish the circumstances favoring diffusion of penicillin into the cerebrospinal fluid.

In this investigation the use of oral caronamide to produce an inhibition of excretion of penicillin by the renal tubules resulted in increased penicillin plasma concentrations and, in the measure that it did so, contributed to the finding of larger quantities of penicillin in the cerebrospinal fluid. The failure of caronamide to pass freely into the cerebrospinal fluid in the majority of patients is another evidence that there is a hindrance of the free interchange of dissolved substances between the blood and the cerebrospinal fluid. The passage of caronamide into the cerebrospinal fluid when excessively high concentrations of caronamide were obtained in the plasma suggests that the barrier is only relatively impermeable.

Although the plasma concentrations of caronamide observed in this study were in excess of the 20 to 40 mg per 100 cc that have been shown to be effective in inhibiting the excretion of penicillin,[27] the only toxic symptoms were nausea and vomiting in 5 patients, and in only 2 patients was it necessary to discontinue administration of the drug.

Conclusions. Therapeutically significant quantities of penicillin (above 0.03 units per cc) have been observed in the cerebrospinal fluid following the intermittent intramuscular injection of 100,000 units of penicillin in aqueous solution. Caronamide increased the plasma levels of penicillin resulting from the administration of this dose of penicillin and thereby increased the amounts of penicillin found in the cerebrospinal fluid. Caronamide itself enters the cerebrospinal fluid only at excessively high plasma concentrations (87 to 110 mg per 100 cc).

The authors wish to express their indebtedness to the Chiefs of the Neurological Services at the Philadelphia General Hospital for their kindness in granting permission to study patients on their services, to Grace Schuchardt for caronamide determinations, and to Elizabeth Hughes and June E. Heckman for penicillin assays.

[27] Boger, William P., Trans. and Studies College of Physicians of Phila., 1947, **15**, 104.

16407

Influence of Dietary Lipids on Experimental Tuberculosis.*

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Experiments are recorded in the literature in which it has been demonstrated that the administration of certain of the lipids may result in an enhancement of experimental tuberculosis while the administration of other of these compounds may result in a retardation of the progress of the infection.

Weigert[1] conducted feeding experiments, using guinea pigs, and concluded that the tuberculous processes generalized more rapidly in animals fed a strict carbohydrate diet than in those receiving an added liberal amount of milk fat.

Troteanu[2] administered cod-liver oil directly into the stomach of guinea pigs and noted an enhancement of the progress of tuberculosis following the inoculation of the animals intraperitoneally with 1 mg of bovine

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[1] Weigert, R., *Berl. Klin. Wechen.*, 1907, **44**, 1209.

[2] Troteanu, V. C., *Comp. Rend. Biol.*, 1929, **102**, 141.

TABLE I.
Saponification and Iodine Values of Body Fat of Groups of Albino Mice Which Had Been Held on Their Respective Rations for a Period of 10 days.

Diet	Saponification value		Iodine value
Non-lipid ration	202.2, 202.3		78.4, 78.3
Coconut oil "	+15.7%*	234.0, 234.0	—12.6%* 68.4, 68.4
Olive " "	0.0%*	202.0, 202.0	+ 7.9%* 84.6, 84.6
Oleic acid "	—1.0%*	201.8, 201.9	+33.9%* 105.0, 105.0
Linseed oil "	0.0%*	202.5, 202.5	+33.9%* 105.0, 105.0
Oils:			
Coconut oil	253.0, 253.0		9.7, 9.7
Olive oil	193.5, 193.5		83.7, 83.7
Oleic acid	195.5, 195.6		91.4, 91.4
Linseed oil	195.0, 195.0		180.0, 180.0

* The percentage increase or decrease in saponification and iodine values of the body fat of animals receiving the coconut oil fatty acids, olive oil, and linseed oil rations as compared with values of the body fat of the animals fed the non-lipid ration.

tubercle bacilli.

Negre[3] reported that the ingestion or subcutaneous injection of cod-liver or olive oil resulted in an enhancement of experimental tuberculosis in guinea pigs and rabbits.

Negre, Berthelot and Bretey[4] injected the ethyl esters of a series of fatty acids subcutaneously into tuberculous guinea pigs. The ethyl esters of palmitic, myristic, lauric, arachidic, capric, and stearic acids retarded the appearance of tuberculous lesions.

The present experiment consists of an investigation of the progress of experimental tuberculosis in groups of mice whose body fat had been changed by the administration of a basal ration containing various types of lipids.

Methods. Male, Swiss albino mice 90 days of age were used in this experiment. The lipids were administered to these animals in the form of oleic acid,† olive oil,‡ linseed oil,§ and the total fatty acid of coconut oil.† Each of the lipids was mixed with a fat-free, casein-supplemented ration[5] in a concentration of 20%. The 4 lipid-containing rations and the non-lipid ration were administered to groups of 25 mice. After having been fed their respective rations for a period of 10 days, 5 animals were removed from each of the 5 groups. These animals served as dietary controls. A second group of 5 animals was selected from each of the groups. The body fat of these animals was utilized for a determination of the saponification and iodine values which are recorded in Table I. The remaining 15 animals in each of the 5 groups were inoculated intravenously with .5 mg of MS-45 strain‖ of *Mycobacterium tuberculosis.* A large inoculum was used since these animals were known to be resistant to experimental tuberculosis.

Average weights of each of the groups of animals were recorded at the time of inoculation. Recordings were also made of the individual weight of each animal at death.

The animals remaining alive at the end of 37 days were sacrificed by exposure to ether vapor. Sections were prepared from the lungs, liver, spleen and kidney tissue of each animal succumbing during the experimental period and from the tissues of those which were sacrificed. Each section was stained with hematoxylin and eosin. Certain of the

[3] Negre, L., *Annal. de l'Inst. Pasteur,* 1932, **49**, 319.

[4] Negre, L., Berthelot, A., and Bretey, L., *Ann. Inst. Pasteur,* 1937, **59**, 457.

† 0-18 "Elaine" and F-716 Coconut Fatty Acids generously supplied by Emery Industries, Inc., Cincinnati, 2, O.

‡ "Torino Brand," J. Ossola Co., New York City.

§ Raw Linseed Oil, U. S. Paint, Lacquer and Chemical Co., St. Louis, Mo.

[5] Pearce, E. L., *et al., Jour. Biol. Chem.,* 1947, **168**, 271.

‖ This strain of *Mycobacterium tuberculosis* was isolated in 1945, from a tuberculous guinea pig which had been inoculated with a sputum specimen originally obtained from Mount St. Rose Sanatorium, St. Louis, Mo.

TABLE II.
Average Weights, Survival, and Average Degree of Pathological Involvement of Tissues of Albino Mice Infected with 0.5 mg of *Mycobacterium tuberculosis*.

	Mice	No. surviving after 37 days	Wt when infected (g)	Wt at death (g)	% of substance occupied by lesions*			
					Lungs	Liver	Spleen	Kidney
Non-lipid ration	15	15†	21.5	19.4	1X	—X	—X	—X
Coconut oil "	15	13†	20.5	17.1	1.3X	1X	—X	—X
Olive " "	15	4†	20.4	13.5	2X	1.3X	2.1X	—X
Oleic acid "	15	5†	20.0	14.1	2.4X	1.3X	1.5X	—X
Linseed oil "	15	4†	19.7	12.3	2.8X	1.5X	—X	—X

* "—X" indicates that less than 25% tissue involvement, "1X" denotes 25%, "2X" 50%, "3X" 75%, and "4X" pathological involvement exceeding 95%.
† Animals sacrificed at the end of 37 days.

sections were also stained by the Ziehl-Neelsen carbol fuchsin technic for acid-fast organisms. The extent of the tuberculous processes in each of the tissues was graded on the following basis: "O," apparently normal; "-X," less than 25% involved pathologically; "1X," 25% involved; "2X," 50% involved; "3X," 75% involved; "4X," exceeding 95% pathological involvement.

The saponification and iodine values indicated that a significant change had occurred in the body fat of the albino mice. In a comparison with the non-lipid ration, the administration of the coconut oil fatty acid ration resulted in a decrease in the iodine value and an increase in the saponification value indicating a deposition of short-chain, saturated acids in the body fat. There was no increase in the saponification values in the case of the olive oil, oleic acid, and linseed oil rations, however, the iodine value increased indicating a deposition of unsaturated acids. The iodine value for the body fat of animals fed the oleic acid ration is inconsistent with the corresponding value for oleic acid. This apparent discrepancy may have been due to the presence in the oleic acid of a saturated and highly unsaturated acid, *e.g.*, stearic and linolenic acid, in which the unsaturated acid was preferentially absorbed from the intestinal tract of the animal.

The histological picture differs from that described by Youmans and McCarter[6] in the extent of the coagulative necrosis occurring in the liver tissue and in the occurrence of tubercles in the medulla of the kidney.

Sections stained by the Ziehl-Neelsen technic revealed the presence of acid-fast bacilli occurring singly or in clumps in all lesions.

The albino mice proved to be resistant to experimental tuberculosis when fed the non-lipid ration. Of the 5 groups of animals, those receiving the non-lipid ration exhibited the maximal survival, a minimal loss of weight, and a minimum of pathological involvement in the lungs, liver, spleen and kidney tissues. Previous experiments indicate that the administration of a non-lipid ration to mice, which are known to be susceptible to experimental tuberculosis, does not change the state of susceptibility of such animals. The observed susceptibility of one strain and the resistance of a second strain of mice when subjected to identical non-lipid dietary regimens, may represent an expression of variation in the native resistance of the species.

The results obtained following the administration of the ration containing the total fatty acids of coconut oil were similar to those which resulted from administering the non-lipid ration. However, there was a slight increase in weight loss and mortality. There was also a slight increase in the pathological involvement of the lungs and liver. The pathological involvement of the spleen and kidney tissues was not different from that of the animals receiving the non-fat ration.

As compared with the non-lipid and coconut fatty acid rations, the administration of the olive oil, oleic acid, and linseed oil rations resulted in a significantly greater mortality,

[6] Youmans, G. P., and McCarter, J., *Amer. Rev. Tuberc.*, 1946, **52**, 432.

TABLE III.
Wt of the Control Series of Albino Mice.

Diet	Avg wt at time of inoculation of test mice	Avg wt after 37 days	Increase in wt (%)
Non-lipid ration	21.4	24.5	12.6
Coconut oil "	18.2	18.5	1.6
Olive " "	19.6	22.0	11.8
Oleic acid "	22.0	24.4	10.9
Linseed oil "	24.2	25.0	3.3

weight loss, and pathological involvement of the lungs, liver, spleen and kidneys. The most extensive lung involvement was observed in those animals receiving the linseed oil ration. The liver and kidney tissue of those animals receiving the olive oil, oleic acid and linseed oil rations displayed an equally severe degree of pathological involvement. The extensive involvement of the spleen tissue of those animals receiving the olive oil and oleic acid rations was striking as compared with the involvement observed in the spleen tissue of the remaining 3 groups of animals.

Approximately equal increases in weight occurred in those animals receiving the non-lipid, the olive oil, and the oleic acid rations. There was little change in the weight of the mice receiving the linseed oil and coconut oil fatty acid rations.

Discussion. It was the purpose of this experiment to effect a deposition of fatty acids in the body fat of the mice corresponding to those of the administered diet. Under such conditions any enhancement or retardation of experimental tuberculosis in the animal could be ascribed to the type of fatty acids present in the body fat. In the case of the non-lipid ration, the fatty acids present in the body fat would be limited to those synthesized from the carbohydrate fraction of the ration.

In a study of the growth of *Mycobacterium tuberculosis* in trypticase[7] and in trypticase-albumin media[8] containing a series of fatty acids,¶ it was found that capric, lauric, palmitic, and myristic acids were inhibitory at concentrations of 10^{-5}% in the former media and at concentrations of 10^{-3}% in the latter media. Growth of *Mycobacterium tuberculosis* occurred at 1% or at 0.1% concentrations of each of the remaining fatty acids of the group tested.

The results of the *in vitro* investigation correlate with the results of this *in vivo* experiment. The administration of the coconut oil fatty acid ration to mice resulted in an enhanced resistance to experimental tuberculosis as compared with the administration of olive oil, oleic acid and linseed oil ration to these animals. Chemical analyses have shown that coconut oil contains approximately 7% capric, 46% lauric, 19% myristic, and 10% palmitic acids. The presence of significant amounts of caprylic, stearic and oleic acids in this oil may be responsible, in part, for the few fatal cases observed in the animals receiving the coconut oil ration.

It has been noted that growth of MS-45 strain of *Mycobacterium tuberculosis* occurs *in vitro* in the presence of 1% concentrations of oleic and linoleic acids. This correlates with the fact that the administration of olive oil and linseed oil rations to mice resulted in increased susceptibility to experimental tuberculosis. The results of the administration of the oleic acid ration was not significantly different from the results obtained by administering the olive oil ration which indicated that oleic acid was the factor responsible for enhanced susceptibility to experimental tuberculosis.

In view of the high percentage of long chain, unsaturated fatty acids composing cod-liver oil, the question arises as to the effect of this

[7] Dubos, R. J., and Davis, B. D., *Jour. Exp. Med.*, 1946, **83**, 409.

[8] *Ibid.*

¶ Formic, acetic, propionic, n-butyric, iso-valeric, iso-caproic, n-heptylic, caprylic, pelargonic, capric, lauric, myristic, palmitic, stearic, oleic, and linoleic acids.

oil, *per se,* on the progress of tuberculous infection.

Schoenheimer[9] has shown that the fatty acids of the diet are first incorporated into body fat before they are oxidized. Thus, the body fat is part of a dynamic system, the fatty acids being continually deposited and withdrawn.

Franke, *et al.*,[10] found that of all the substrates tested manometrically, certain of the fatty acids effected the greatest increase in respiration of *Mycobacterium tuberculosis* despite the fact that other of these acids were inhibitory under such conditions.

Due to the fact that *Mycobacterium tuberculosis* is located extra-cellularly in its host, this microorganism has access to the body fluids of the host. Since the body fat is in a dynamic state, such fluids will contain fatty acids corresponding to those of the diet. If the diet contains large amounts of fatty acids which serve to increase the respiration of *Mycobacterium tuberculosis,* enhanced proliferation of the organism would be expected to occur. However, if the diet contains large amounts of fatty acids which serve to inhibit the respiration of *Mycobacterium tuberculosis,* a retarded proliferation of the organism would be expected to result.

Thus, the type of fatty acids present in the diet may determine whether or not conditions are favorable for the development of tuberculous infection.

Summary. 1. The administration of a non-lipid, casein-supplemented ration to resistant Swiss albino mice retarded the progress of experimental tuberculosis. 2. Experimental tuberculosis was also retarded by the administration of the ration to which 20% of the total fatty acids of coconut oil had been added. 3. Olive oil, linseed oil, and oleic acid enhanced the progress of experimental tuberculosis when these compounds were added to the ration in a concentration of 20%.

[9] Schoenheimer, R., and Rittenberg, D., *Jour. Biol. Chem.*, 1936, **114**, 381.

[10] Franke, W., and Schillinger, A., *Biochem. Z.*, 1944, **316**, 313.

16408

A Lipid Anticoagulant from Brain Tissue. Physiochemical Characteristics and Action *in vitro* and *in vivo.**

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Dilution of a crude aqueous brain extract or of a suspension of its lipid (cephalin) fractions often improve their clot accelerating power. This apparent paradoxical behavior of strong thromboplastic solutions has been frequently observed.[1,2,3] Attempts to separate the clot inhibiting from the clot accelerating substances have only been moderately successful.[3,4] The experiments here reported indicate that it is possible to separate from brain tissue, by a suitable method of extraction, a heat labile inhibitor of blood coagulation, probably of a lipid nature and acting *in vitro* and *in vivo* as a powerful antithromboplastin.

Reagents. Thromboplastin. 300 mg of acetone dried human brain powder are extracted for ½ hour with 5 cc of 0.85% sodium chloride at 50°C. The supernatant fluid is centrifuged at 1000 R.P.M. for 2 minutes and tested against recalcified citrated plasma.

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[1] Aggeler, P. M., and Lucia, S. F., *Am. J. Med. Sci.*, 1940, **199**, 181.

[2] Kazal, L. A., and Arnow, L. E., *Arch. Biochem.*, 1944, **4**, 185.

[3] Chargaff, E., *J. Biol. Chem.*, 1937, **121**, 175.

[4] De Suto Nagy, G. J., *J. Biol. Chem.*, 1944, **156**, 433.