gelates completely, incubation for 30 min is necessary, since at temperatures of 20 to 25° C maximal firmness may not develop within 1 hr. It is of interest that in hemophilia both the clot resistance[5] and the clot firmness are decreased. Blood clots from 2 heparinized rabbits exhibited firmness equal to 10 to 40 mm of Hg which was not affected by 2 hr of incubation.

An exploratory study on clot firmness was done on blood clots of a "dicoumarinized" patient with a history of recurrent thrombophlebitis. Following therapy with 3,3′-methylenebis (4-hydroxycoumarin) the coagulation time and prothrombin time were prolonged, whereas the bleeding time and clot resistance were normal. The clot firmness equalled 70 mm of Hg after 40 min incubation and increased to 200 mm of Hg by 80 min. This phenomenon of an increase in clot firmness after 30 min incubation was observed on 3 separate occasions in blood clots from this patient. We believe this could be due to a delayed formation of fibrin, although it is possible that the change might occur subsequently in the fibrin itself. These findings, and those in normal animals, indicate that a clot which forms in a tube continues to increase in firmness for a period of time.

[5] Copley, A. L., and Lalich, J. J., *J. Clin. Invest.*, 1942, **21**, 145.

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Influence of Essential Fatty Acid Deficiency on Transport of Fatty Acids into the Liver.*

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Although it is well recognized that linoleic acid is not synthesized by the animal body and is a dietary essential for several species of laboratory animals there is no evidence as to the metabolic function of this fatty acid. The common observation that linoleic acid is a normal and apparently ever-present constituent of tissue phospholipids led Burr and Burr[1] to consider the possibility that linoleic acid is necessary for the normal metabolic functioning of tissue phospholipids. The nature of the functions of phospholipids is not definitely known, but one well recognized characteristic is the exchange of phosphorus and fatty acids in the phospholipid molecule. Two attempts have been made to study the rate of phospholipid turnover in the tissues of rats suffering from a deficiency of the essential fatty acids, by Hevesy and Smedley-MacLean,[2] using radioactive phosphorus as a tracer, and by Barnes, Miller, and Burr,[3] using labeled (spectroscopically active) fatty acids. In the labeled acid study essential fatty acid deficiency resulted in a slight decrease in the rate of fatty acid incorporation into the phospholipids of the intestinal mucosa, hence it was thought desirable to extend this type of study to other tissues. The present investigation was undertaken to study fatty acid incorporation into the liver phospholipids of rats subjected to a fat deficient regimen.

Details of the various methods employed

* Assistance in the preparation of these materials was furnished by the personnel of the Works Projects Administration, Official Project No. 265-1-71-236, Subproject 612.

[1] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1930, **86**, 587.

[2] Hevesy, G. C., and Smedley-MacLean, I., *Biochem. J.*, 1940, **34**, 903.

[3] Barnes, R. H., Miller, E. S., and Burr, G. O., *J. Biol. Chem.*, 1940, **140**, 773.

TABLE I.
Rate of Incorporation of Labeled Fatty Acids into the Acetone Insoluble (Phospholipid) Lipid Fraction of the Livers of Essential Fatty Acid "Deficient" and "Cured" Rats.

Treatment	Absorption time hrs	No. of rats	Body wt. g	Wt. of liver g	Phospholipid in liver %	$E^{1\%}_{1cm}$ of phospholipids	Labeled fatty acids in phospholipids %
Cured of F.D.	0	7	160	4.45	3.64	10.3	
F.D.	0	6	126	3.95	3.12	7.9	
Cured of F.D.*	8	3	158	4.95	3.17	8.0	
F.D.*	8	3	133	4.30	3.59	8.0	
Cured of F.D.	2	7	162	4.65	3.45	15.7	1.5
F.D.	2	7	129	4.00	3.54	12.3	1.2
Cured of F.D.	8	7	160	4.84	2.72	23.2	3.6
F.D.	8	7	131	4.28	3.19	21.0	3.6
		Controls from stock diet.†					
Normal	0	4	212	4.29	2.82	28.2	
	8	4	207	5.02	2.87	42.3	3.9

*Rats in these two groups received the normal, unconjugated corn oil (Mazola) having an $E^{1\%}_{1cm}$ at 2350 A of approximately 3.0.

†Data from reference 5, p. 250, Table I, Exp. 3.

have been described elsewhere.[4, 5] The conjugated fatty acids of corn oil, prepared as the methyl esters, were employed as the labeled fatty acids. These esters had an extinction coefficient, $E^{1\%}_{1cm}$ at 2350 A, that was 50 times greater than that of the normal liver acetone insoluble lipid fraction. Rats that had been fasted for 24 hours were fed by stomach tube 0.5 cc of the fatty acid esters per square decimeter body surface.[6] After intervals of 2 and 8 hr the rats were killed by etherization. The livers were weighed, ground in a mortar with sand and finally covered with an alcohol-ether mixture (3:1). Lipid extraction was carried out for several hours at boiling temperature under a stream of nitrogen, the alcohol-ether extract was evaporated almost to dryness and residue taken up in petroleum ether. This extract was washed several times with water, and after a partial evaporation the phospholipids were precipitated with acetone. The acetone insoluble lipids were then taken up in moist ether and analyzed spectroscopically for their content of labeled fatty acid. Details of this analytical procedure have been described.[4]

Two separate experiments were carried out in which female weanling rats were placed on a diet composed of casein 12%, sucrose 84.1%, and salts 3.9%,[7] supplemented by daily doses of 0.7 g of dried yeast and concentrates of vitamins A, D, and E.[8] At the end of 3 months when the growth curves had almost reached a plateau, one half of the rats were given a daily supplement of 3 to 4 drops of corn oil (Mazola) for one month in order to bring about a cure of the deficiency symptoms. The remaining rats continued on the fat deficient regimen during this time. Although definitely improved as to growth and skin conditions none of the rats was com-

[4] Miller, E. S., Barnes, R. H., Kass, J. P., and Burr, G. O., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 485.

[5] Barnes, R. H., Miller, E. S., and Burr, G. O., *J. Biol. Chem.*, 1941, **140**, 247.

[6] Carman, G. G., and Mitchell, H. H., *Am. J. Physiol.*, 1926, **76**, 380.

[7] McCollum, E. V., and Simmonds, N., *J. Biol. Chem.*, 1918, **33**, 63.

[8] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

pletely cured by this treatment, and for comparative purposes a control group of female rats which had been raised on a stock diet was included in the summary of data presented in Table I. The data for this added control group were taken from a previous publication.[5]

No significant difference exists between the fat deficient and the "cured" animals in the rate of entrance of labeled acids into the acetone insoluble fraction of the liver lipids. Furthermore, the small difference between the two experimental groups and the controls on stock diet is probably well within experimental error.

It is still possible that fatty acid turnover in some tissue phospholipids is affected by essential fatty acid deficiency. Hevesy and Smedley-MacLean[2] found that rats receiving a fat deficient diet showed the same relative turnover of radioactive phosphorus in the kidney and liver phospholipids but greater in muscle phospholipids than did rats which had been cured of the deficiency with linoleic or arachidonic acids. Barnes, Miller, and Burr[3] found a decreased exchange of labeled fatty acids in the intestinal mucosa phospholipids of fat-deficient rats. Until more is known about the metabolic role of this fatty acid exchange phenomenon in phospholipids, the significance of small deviations from normal must be a matter of conjecture.

Summary. Rats suffering from a severe deficiency of the essential fatty acids show the same rate of incorporation of labeled fatty acids into the liver phospholipids as do rats which have been cured of this deficiency by the administration of 3 to 4 drops of corn oil for 1 month.

The rate of incorporation of labeled acid into the liver phospholipids is the same for rats raised on the fat deficient diet as has been previously observed for rats receiving a regular mixed stock diet.

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Production of the Shwartzman Phenomenon with a Sulfonamide Conjugate of a Bacterial Filtrate.*

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A bacterial filtrate* of meningococcus, previously shown to be capable of producing the Shwartzman phenomenon,[1] was coupled with paraaminobenzenesulfonylacetylimide† by means of a modification of the original Landsteiner method.[2] The resulting conjugate was a highly colored substance, russet and soluble in alkalis, yellow and insoluble in acids. The conjugate was purified by 10 reprecipitations from sodium carbonate solution using dilute acetic acid as the precipitating agent. The final precipitate was suspended in normal saline and dissolved by neutralization with normal sodium carbonate.

Eight experiments were performed on rabbits. In 4 instances the skin was prepared by an intradermal injection of 0.5 cc of the original bacterial filtrate and in the other 4 by the conjugate. A single intravenous injection of 1.0 cc of either the filtrate or the conjugate was given 24 hours later in each instance. Two hours later a hemorrhagic reaction was observed at the site of skin

* Courtesy of Dr. Gregory Shwartzman, Department of Bacteriology, Mount Sinai Hospital, New York City.

† We are indebted to the Medical Research Division, Schering Corporation, Bloomfield, for this compound.

1 Shwartzman, G., *The Phenomenon of Local Tissue Reactivity,* Paul B. Hoeber, Inc., Med. Book Dept., Harper and Brothers, New York, 1937.

2 Landsteiner, L., and Lampl, H., *Z. Immunitaet. Forsch.,* 1917, **26**, 293.