of an antigen such as BCG is not surprising in view of the fact that this species is known to respond only slightly, or not at all, to most antigenic or allergenic stimuli. It is possible that the unusual lack of response of the rat to BCG immunization is another reflection of its peculiar biological behavior and may be in some way related to its native resistance to infection.

The increase in gamma globulin during BCG immunization in the rabbit and guinea pig follows the classical relation of an increase in gamma globulin with the production of antibody(13,14). The extra-normal T component sometimes seen to occur as a result of certain antigenic stimulation(15) was not observed in this study.

The comparative protein response of the natively resistant rat and susceptible rabbit and guinea pig to tuberculous infection is now under investigation in this laboratory.

Summary. Total serum protein concentration and electrophoretic distribution of the serum protein components of the rabbit, guinea pig, and rat were determined by a paper electrophoretic technic. The serum protein was again analyzed 4 weeks after the animals were inoculated with an attenuated strain (BCG) of tubercle bacilli. Statistically significant increases in the total protein and gamma globulin occurred in the rabbit and guinea pig, but not in the rat. A decrease in the alpha-1 globulin in the rat and an increase of this fraction in the guinea pig was observed following BCG administration. Significant alterations in the other protein fractions were not observed in any of the 3 species of animals.

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Oxidation of Lecithin and Sphingomyelin by Tissue Preparations.* (22389)

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The metabolic functions of the phospholipids are not clearly understood at the present time. Liver(1-3) and liver mitochondria (3) are known to contain dehydrogenases which act on purified phospholipids. However, the ability of other tissues to attack phospholipids has not been tested. Accordingly in the present experiments the phos-

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pholipid dehydrogenase activity of homogenates of brain, liver, skeletal muscle, spleen, and intestinal mucosal was assayed with purified lecithin and sphingomyelin as substrates. The cofactors added in these experiments were hypoxanthine, ATP, or DPN, since these compounds have been found necessary for known phospholipid(1-3) or fatty acid dehydrogenases(4-6).

Methods. The sources and purity of the phospholipids and other chemicals used in this study have been previously described(3). Homogenates of rat skeletal muscle were prepared by mixing 5 g of rat spinal muscle with 50 ml of 0.25 M sucrose for 1 minute at 0° in the 125 ml cup of the Waring blendor at ⅓ of full speed. Homogenates of other tissues were made by grinding 1 part of finely divided tissue with 2 parts of 0.25 M sucrose in a motor driven all glass homogenizer(7). All preparations were filtered through fine cheese cloth before use. Acetone powders were prepared in the usual manner from suspensions of liver mitochondria in 0.25 M sucrose or from homogenates of intestinal mucosa. Extracts of the acetone powders were made by grinding 1 part of fresh powder with 3 volumes of isotonic sucrose and centrifuging at 18,000 G at 0° for 30 minutes. Rat liver mitochondria were prepared by the method of Kielley and Kielley(8) except in the cases of those organelles used for acetone powders. The mitochondria used for the latter purpose were isolated on a large scale by the method of Witter, Pories, and Cottone (9). In each case the particulates were suspended in enough 0.25 M sucrose so that 1 ml of suspension was equivalent to the mitochondria from 0.5 g of liver. In a total volume of 1 ml the complete reaction mixture contained 0.1 mg of substrate, 0.01 M phosphate buffer pH 7.4, 0.025 M sucrose, 0.01 M sodium malonate, 0.01 M potassium cyanide, 0.005% 2,6-dichlorobenzenoneindo-3′-chlorophenol, either 0.001 M ATP or 1 x 10^{-4} M DPN, and either 0.4 ml of homogenate of rat spinal muscle or 0.1 ml of the homogenate of other tissues or extracts of acetone powders. In some experiments 0.005 M magnesium chloride was added. Under anaerobic conditions 0.0033% methylene blue could replace the indophenol dye plus cyanide. The dehydrogenase activity was estimated by comparing the rate of reduction of the dye in a tube containing the complete reaction mixture with that in another tube containing a similar mixture from which the substrate had been omitted. The reduction of the dye was followed for 1 hour.

Results. In Table I are given the results of the studies of the phospholipid dehydrogenase activity of homogenates of spleen, muscle, intestinal mucosa and suspensions of mitochondria from rat liver, using hydrolecithin or sphingomyelin as substrates. For purpose of brevity data showing that the systems are inactive in the absence of added DPN or ATP have been omitted. It can be seen that at least 2 enzymes or enzyme systems were detected. The first of these, a dehydrogenase requiring ATP, was found in liver mitochondria as previously described(3) but in addition was observed in spleen. Other experiments showed that the dehydrogenase of mitochondria was inhibited by the concentration of magnesium chloride employed whereas the enzymes in the other homogenates were not. The second type of dehydrogenase required DPN and was present in muscle, intestinal mucosa, and spleen. The enzyme in the latter tissue may be different from the one present in the intestinal mucosa or muscle since the dehydrogenase of spleen acted only on sphingomyelin while that of intestinal mucosa or muscle acted either on lecithin or sphingomyelin. Other experiments not given in Table I showed that homogenates of whole liver did not reduce the dye at an increased

TABLE I. Distribution of Phospholipid Dehydrogenases.

Assay system ATP	DPN	Substrate	Reduction time in min. Liver mitochondria	Spleen	Muscle	Intestine
+	—	0	11	>60	21	30
+	—	Hyd†	3	9	24	25
+	—	Sph	6	>60	23	>60
—	+	0	8	>60*	19*	14
—	+	Hyd	9	>60*	6*	6
—	+	Sph	8	3*	9*	7

* Magnesium chloride was present.
† Hyd = Hydrolecithin; Sph = Sphingomyelin.

rate in the presence of DPN and that neither the dehydrogenase which required DPN nor the one for which ATP is a cofactor could be detected in homogenates of brain or kidney. The only tissue in which the phospholipid dehydrogenase which requires hypoxanthine(1, 2) could be detected was liver, and in confirmation of previous work(3) this dehydrogenase was not present in the liver mitochondria.

The phospholipid dehydrogenase of intestinal mucosa was found to be present in an acetone powder of this tissue as had previously been reported for the enzyme of rat liver mitochondria(3). In addition the phospholipid dehydrogenase of liver which required hypoxanthine was inactivated by treatment with acetone. Thus acetone powders of whole liver could also be used as a source of the mitochondrial enzyme. As is shown in Table II the enzymes could be obtained in soluble form from the acetone powders.

In the next experiment the specificity of the phospholipid dehydrogenase present in the extracts of acetone powder of intestinal mucosa or liver mitochondria was investigated. Oleic acid, lecithins containing both saturated and unsaturated acids, and lipids containing sphingosine such as sphingomyelin, cerebroside, or ceramide were active. Evidently the action of these enzymes is not limited to the catalysis of dehydrogenation in the 9, 10 position of the fatty acid moiety. It is of interest that the synthetic lipid glycolecithin which has not been found in natural products was attacked by the enzyme system.

TABLE II. Extraction of Phospholipid Dehydrogenase from Acetone Powders of Liver and Intestine.

	Reduction time in min.			
	Intestine		Liver mitochondria	
Enzyme source	Endogenous	Hydrolecithin	Endogenous	Hydrolecithin
Original powder	12	5	10	5
Residue from centrifugation	>60	>60	>60	>60
Supernatant	29	11	18	7
Dialyzed supernatant	>60	>60	>60	>60

TABLE III. Specificity of Phospholipid Dehydrogenase in Extracts of Acetone Powders of Liver Mitochondria and Intestine.

	Reduction time in min.	
Substrate	Liver mitochondria	Intestine
None	13	16
Sphingomyelin	7	9
Ceramide	8	10
Palmitoleyl lecithin	4	7
Hydrolecithin	4	7
Cerebroside	7	10
Oleic acid	4	10
Palmitic acid	3	10
Glycolecithin	9	11

The results of these studies show that the rate of reduction of indophenol dyes by homogenates of various tissues is increased by the presence of lecithin and sphingomyelin. The exact nature of the reaction which causes the reduction of the dye is not revealed by these experiments. Rodbell and Hanahan(10) have shown that small amounts of lecithin and its derivatives will catalytically stimulate the respiration of liver homogenates supplemented with acids of the Krebs cycle. However, this does not seem to be the case in the present experiments since the endogenous respiration was repressed with malonate, since specific cofactors were required and since no increase in the rate of reduction was noted if the level of phospholipid was reduced to values less than ¼ that usually employed. It is more probable that the increased rate of reduction of the dye in the present experiments is a measure of the oxidation of either the intact phospholipid or its hydrolysis products. In view of the differences in the effect of the presence of magnesium chloride and in the requirement for activating factor for each tissue it appears probable that the reactions measured by the phospholipid dehydrogenase system are not the same in each tissue.

Investigation of the products of these reactions by means of paper chromatographic methods for phospholipids and their hydrolysis products which were recently developed in this laboratory(11) may reveal the exact pathway by which the phospholipids are attacked in each system. The present experiments have provided a basis for the further study of the oxidation of phospholipids *in*

vitro since four tissues worthy of further investigation have been ascertained, and the active enzymes have been obtained in soluble form from two of them.

Summary. Liver mitochondria and spleen were found to contain dehydrogenases which attacked lecithin and sphingomyelin and required ATP. Muscle and intestinal mucosa had dehydrogenase activity with lecithin or sphingomyelin if DPN was present whereas under the same conditions homogenates of spleen were active only with sphingomyelin. The dehydrogenase systems were obtained in soluble form from acetone powders of liver mitochondria or intestinal mucosa.

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Phenylserine Studies. IV. Biochemical Activity of *o*- and *p*-Fluorophenylserine. (22390)

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Phenylserine was shown(1) to be an inhibitor of the growth of various species of bacteria. The inhibition was associated with certain enzymatic sequences in phenylalanine and tryptophan metabolism, since either of these two amino acids was able to reverse the inhibitory effect. Subsequently, the discovery of chloromycetin, which may be considered to be a phenylserine derivative, has stimulated increased interest in the bacteriostatic action of this type of compound(2). Fluoro-derivatives of various metabolites have frequently proven of interest in the study of metabolic sequences, presumably because the small atomic dimension of the fluorine atom does not prevent the reaction of the analogue with an enzyme, but does prevent completion of the enzymatic sequence. The fluoro-analogues of phenylalanine have previously been prepared and tested as to their bacteriostatic properties(3,4). It therefore seemed of interest to synthesize and test the properties of ring-substituted fluoro-derivatives of phenylserine. The present paper describes the microbiological effects of the *o*-fluoro- and *p*-fluoro- analogues of phenylserine.

Materials and methods. Fluorophenylserines. Into an evaporating dish are introduced 19.8 ml of a 2.5 M glycine solution, 9.9 ml of 95% ethyl alcohol, 9.0 ml of *o*- or *p*- fluorobenzaldehyde,* and 19.8 ml of 6.25 M sodium hydroxide. The mixture is stirred until it becomes clear, permitted to stand for one hour or until a waxy white precipitate forms, and the precipitate dried between sheets of filter paper. The precipitate is washed twice with 15 ml portions of ethanol and then twice with 10 ml portions of water. To the residue is added 500 ml of water with stirring, acetic acid is added to pH 6.0, and any residue is filtered off and discarded. The filtrate is evaporated to dryness, taken up in hot water, and recrystallized from the water

* Obtainable from Custom Chemical Laboratories, Chicago, Ill.