terial, and to avoid the loss incurred during lyophilization by immediately subjecting the product to electrophoresis on blocks of cellulose were not successful in the limited time for exploration of these points.

Discussion. Although the activities of the final products were approximately 230 times that of Mylase P on the basis of purification of protein, the activities were approximately 1900 times that of Mylase P on a gravimetric basis since the starting material contained only 12% protein. In spite of these extensive purifications, the results indicate that fungal phenolsulfatase was not pure by the criterion of paper electrophoresis at pH 6.7. The retention of most of the enzymatic activity during electrophoresis, however, suggests that electrophoresis on blocks of cellulose might be suitable for further purification of larger amounts of the enzyme.

Summary. Fungal phenolsulfatase of Mylase P was purified by fractionations with ethanol, zone electrophoresis on cellulose, and treatment with aluminum hydroxide gel. Related to the activity of the original Mylase P the enzyme was purified approximately 1900 times. On the basis of purification of the protein of Mylase P approximately 230-fold purifications were achieved.

1. Neuberg, C., Kurono, K., *Biochem. Z.*, 1923, v140, 295.
2. Fromageot, C., *Ergeb. Enzymforsch.*, 1938, v7, 50.
3. ———, *In the Enzymes,* Academic Press, N. Y., 1950, v1, Part I, 517.
4. Abbott, L. D., *Arch. Biochem.*, 1947, v15, 205.
5. Cohen, H., Bates, R. W., *Endocrinology,* 1949, v44, 317.
6. ———, *ibid.*, 1949, v45, 86.
7. Katzman, P. A., Straw, R. F., Buehler, H. J., Doisy, E. A., *Recent Progress in Hormone Research,* 1954, v9, 45.
8. Lowry, O. N., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.*, 1951, v193, 265.
9. Noelting G., Bernfeld, P., *Helv. Chim. Acta.*, 1948, v31, 286.
10. Sumner, J. B., *J. Biol. Chem.*, 1924, v62, 287.
11. Huggins, C., Smith, D. R., *ibid.*, 1947, v170, 391.
12. Burkhardt, G. N., Wood, H., *J. Chem. Soc.*, 1929, 141.
13. Cohn, E. J., Strong, L. E., Hughes, W. L., Jr., Mulford, D. J., Ashworth, J. N., Melin, M., Taylor, H. L., *J. Am. Chem. Soc.*, 1946, v68, 459.
14. Kunkel, H. J., Slater, K. J., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 42.
15. Marshall, J., Welker, W. H., *J. Am. Chem. Soc.*, 1913, v35, 820.
16. Sorensen, S. P. L., *Ergeb. Physiol.*, 1912, v12, 393.

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Biosynthesis of Cholesterol in Animal Blood.* (24410)

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Investigations were carried out on biosynthesis of cholesterol from C^{14} labelled sodium acetate, in human leukemic blood, and an apparently high rate of synthesis was observed in blood from patients suffering from myelogenous leukemia (unpublished data). Keeping this in mind, an attempt was made to find a good source of the enzyme system responsible for biosynthesis of cholesterol in blood. Hence, blood samples from different animals like rat, rabbit, pig, piglet, calf and cow were tried. Results with calf blood seem to be very encouraging.

Materials and methods. Blood samples from cows, calves and pigs were procured from local slaughter houses. Calf blood was taken from very young animals, 3 to 5 years old. Five calf blood samples were analyzed in 2 separate sets of 3 and 2 each. Adult cows and pigs, Sprague-Dawley strain adult male rats (300-400 g weight), and New Zealand strain adult male rabbits (6 lb weight) were used for their respective blood samples. Blood samples from rats, rabbits and piglets

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TABLE I. Incorporation of C^{14} Labelled Sodium Acetate into Cholesterol in Animal Blood.

Animal	Sample	Sex	Free cholesterol, mg %	Counts/mg/min. of: Fatty acids	Cholesterol from digitonide	Digitonide mother liquor	Cholesterol from dibromo derivative
Calf	1	♀	74	2198	2520	5454	1396
	2	♀	110	11037	999	1250	435
	3	♂	75	5072	1671	2641	723
	4	♂	121	16849	1056	1556	433
	5	♂	92	6269	1181	2095	409
Cow	1	♀	104	26	100	109	41
	2	♀	105	45	145	112	70
	3	♀	71	1166	110	226	45
Pig	1	♀	190	3115	323	8280	209
	2	♀	164	1698	651	4055	175
	3	♂	185	3821	486	977	324
Piglet	1	♀	128	947	90	477	50
	2	♂	108	412	129	237	65
Rabbit	1	♂	73	423	120	451	45
	2	♂	124	230	285	336	39
	3	♂	62	225	45	295	7
Rat	1	♂	112	2204	164	1163	59
	2	♂	123	1368	52	792	25
	3	♂	102	1054	61	1027	19
			Control experiments (heat denatured blood)				
Cow		♀	51	nil	nil	4	nil
Rat		♂	75	15	nil	5	nil

were taken by heart puncture of the animals while under light ether anesthesia. The rat blood sample (40 ml) for incubation was made up by pooling blood from 5 rats. Piglet blood samples were taken from very young animals, 7 days old, and weighing about 7 lbs. Forty ml of citrated blood sample was oxygenated for 20 minutes, with continuous agitation, using a mixture of 95% O_2 and 5% CO_2. Then it was incubated with 800 units penicillin G, 4 mg streptomycin sulfate, and 16 microcuries C^{14} labelled sodium acetate representing 0.185 mg Na acetate, at 37° C, for 6 hours. Samples were hydrolyzed for 16 hours with potassium hydroxide (30% by weight final concentration) and alcohol (one-fifth volume). The hydrolysates were acidified with concentrated hydrochloric acid, and extracted with 2 volumes of pentane-ether mixture (1:1) 3 times. The extract was washed with water, and the fatty acids separated by washing the pentane-ether extract with 10% KOH 3 times. The pentane-ether extract was washed with water till it was free of alkali, and was then evaporated to dryness. The cholesterol from this dry residue was obtained by precipitating it with digitonin, and splitting the digitonide as described by Schoenheimer and Dam(1). The cholesterol from digitonide was purified by dibromo method as described by Fieser(2). KOH washings containing fatty acids were acidified and extracted with pentane. For radioactive count on samples, the dry residues were dissolved in chloroform, and aliquots (containing about 1 mg) were put in the form of a very thin film on aluminum planchets, counted in a flow gas Geiger-Muller Counter. Free cholesterol values were obtained by working up an aliquot of the blood sample by the Sperry-Webb method(3). The red blood cells and white blood cells were counted as described by Kolmer and Boerner(4). Blood smears were also made for the differential white cell count.

Results. From the last column of Table I, it is evident that decisively positive incorporation of C^{14} labelled acetate into cholesterol took place in calf and pig blood samples. The incorporation was more in calf blood samples than in pig blood samples. Sex of animal does not seem to have any influence on incorporation of acetate into cholesterol. A fairly high count of radioactivity in fatty

TABLE II. Blood Picture of Animals.

				Percent differential leukocyte count												
				Neutrophils				Lymphocytes				Monocytes				
Animal	Sample	Erythros, millions	Leukocytes, thousands	Segs	Bands	Juve	Lympho-blasts	Norm	Atyp	EOS	Baso	Norm	Atyp	Histio	Normo-blasts	Megalo-blasts
Calf	1	7.40	6.5	36	9	7		42	2	4						
	2	9.61	8.4	19	4			77								
	3	8.12	9.6	35	16		2	41	5			1				
	4	7.12	7.4	3				91	5	1						
	5	6.90	9.5		5			84		11						
Cow	1	7.04	11.4	64	10	2		18		4	2					
	2	6.24	7.5	35	24	17		14	2	7		1				
	3	6.87	10.6	58	9	2		22	4	1		4				
Pig	1	6.17	16.2	20	5			68		7						
	2	8.29	19.3	21	4	1		67	1	6						
	3	7.30	18.7	20	2			69		9						
Piglet	1	10.00	15.4	9	12	4		64	5	3					3	
	2	8.57	14.4	17	17	1		52		2					11	
Rabbit	1	5.90	11.1					23	21	37		2	12	1	3	1
	2	5.92	8.6					21	34	34			18	3		
	3	6.59	10.0					60	13	18		3	4	1		
Rat	1	7.69	13.2	7	2			87	3	1						
	2	8.66	8.9	17	2			77		3			1			
	3	9.45	9.7	18				68	9	2		2		1		

acids in all samples, indicates incorporation of radioactive acetate into blood fatty acids, regardless of the species. This is observed with human blood also (unpublished data). In all experiments except one, the radioactive count in digitonin cholesterol samples is higher than that in dibromo cholesterol samples, but is lower than that in digitonide mother liquor residues. This is in accordance with the observations of Schwenk *et al.*(5), who described the presence of cholesterol precursors in digitonin cholesterol samples and also in digitonide mother liquor residues.

It is very interesting to note that calf blood has a very active enzyme system for biosynthesis of cholesterol, while this activity is markedly decreased as the animal attains adulthood, as is evident from the results with cow blood. This pattern, however, is not observed with pig and piglet blood samples. We observed that a positive incorporation of acetate into cholesterol takes place in human myelogenous leukemic blood (unpublished data). Since leukemia is a disease of leukocytes, it was thought worthwhile to investigate the differential white cell pattern in blood of these species of animals (Table II). These observations, however, do not help in explaining the chemical findings, since total and the differential leukocyte counts of calf and pig blood samples, in which positive incorporation of acetate into cholesterol was observed, did not differ significantly or regularly from those of others. A very low radioactive count in cholesterol synthesized by rats, rabbits, piglets and cows as compared to complete lack of radioactivity in cholesterol in control experiments carried out with heat denatured cow and rat blood samples (Table I), indicates existence of the enzyme system responsible for biosynthesis of cholesterol from acetate in blood of these animals. Decisive incorporation of acetate into cholesterol in calf and pig blood samples strengthens the evidence in support of the existence of the enzyme system responsible for biosynthesis of cholesterol from acetate, in animal blood.

Summary. A study of biosynthesis of cholesterol *in vitro* from radioactive acetate in animal blood was undertaken. Degree of incorporation of acetate into cholesterol was higher in calf blood than in pig blood samples. Cholesterol synthesized in rat, rabbit, cow, and piglet blood samples had comparatively a very low radioactivity.

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1. Schoenheimer, R., Dam, Z. phys*iol. Chem*, 1933, v215, 59.

2. Fieser, L. F., *J. Am. Chem. Soc.*, 1953, v75, 5421.

3. Sperry, W. M., Webb, M., *J. Biol. Chem.*, 1950, v187, 97.

4. Kolmer, J. A., Boerner, F., *Approved Laboratory Technic*, D. Appleton Century Co., 1945, 57, 66.

5. Schwenk, E., Todd, D., Fish, C. A., *Arch. Biochem. and Biophys.*, 1954, v49, 187.

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Curarizing Effect of Neomycin and its Fractions in the Frog.* (24411)

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It is well known that drugs show multiplicity of action in clinical use. Neomycin, reported by Waksman(1) as effective antibiotic agent against streptomycin-resistant bacteria, can produce prolonged postoperative hypopnea or apnea when administered intraperitoneally in man(2-9). An attempt was made in this study to determine the mechanism of this respiratory depression and whether neomycin or its fractions A (Neamine), B and C possesses a curare-like action.

Methods. 67 adult frogs were employed. *In vivo* sciatic nerve-gastrocnemius muscle

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