endogenous cholesterol on cholesterol synthesis. This report describes these findings (*4*).

Rats with a thoracic lymph fistula were prepared and given saline to drink. Twenty-four hours after the operation, the lymph-fistula animals and normal fasted rats were given intraperitoneally a tracer dose of 30 μc of 1-C^{14}-acetate. The animals were sacrificed at 1 hour, and the liver and blood were removed. The total cholesterol was isolated by extraction, hydrolysis, and digitonin precipitation (*5*). The digitonides were plated and C^{14} activity was determined in a windowless gas flow counter and corrected for self-absorption.

The results, expressed as specific activity of the isolated cholesterol, are shown in Table 1. There was an eightfold increase in the specific activity of the liver cholesterol of the lymph-fistula rat over that of the normal rat. In the plasma, the specific activity of the cholesterol of the lymph-fistula rat was approximately 13 times that of the normal rat. In addition, there was no significant change in the total cholesterol content of the liver and plasma in the lymph-fistula animal.

The amount of cholesterol present in a 24-hour sample of lymph from a fasting rat is 8 to 10 mg. This represents sterol derived from the hepatic lymph and from reabsorption of cholesterol secreted in the bile and from the intestinal mucosa. It appears that the removal of this amount of endogenous cholesterol (8 to 10 mg) during a 24-hour fast leads to a pronounced increase in the rate of cholesterol synthesis in the liver. Moreover, the ratio of the specific activity of the plasma cholesterol to that of the liver cholesterol in the two types of animals suggests that there is a more rapid release to the plasma of newly synthesized cholesterol in the lymph-fistula animals.

These findings provide further evidence that the homeostatic control of cholesterol synthesis is sensitive to fluctuations in the cholesterol supply. Exogenous cholesterol depresses synthesis; in the present experiments, the "bleeding out" of 8 to 10 mg of endogenous cholesterol by way of the lymph fistula produced a marked increase in synthesis. Since exogenous cholesterol and a part of the endogenous supply of cholesterol enter the plasma by way of the thoracic duct, a major factor controlling the rate of cholesterol synthesis in the liver may be the amount of cholesterol entering the plasma by this lymph route. These experiments also pose a question whether the removal of endogenous cholesterol would be effective in lowering the levels of blood and tissue cholesterol.

Table 1. Liver cholesterol synthesis in 1 hour from 1-C^{14}-acetate in normal and lymph-fistula rats. There were four animals in each group.

Group	Specific activity (count/min mg of cholesterol)		Total concn. of cholesterol	
	Liver	Plasma	Liver (%)	Plasma (mg %)
Normal	359 ± 142	128*	0.219	58
Lymph fistula	2935 ± 293	1627 ± 173	0.212	54

* Plasma was pooled.

LEON SWELL, E. C. TROUT, JR., HENRY FIELD, JR., C. R. TREADWELL
Veterans Administration Center, Martinsburg, West Virginia, and *Department of Biochemistry, George Washington University School of Medicine, Washington, D.C.*

References and Notes

1. G. M. Tomkins, H. Sheppard, I. L. Chaikoff, *J. Biol. Chem.* 201, 137 (1953); R. G. Gould and C. B. Taylor, *Federation Proc.* 9, 179 (1950).
2. J. T. Van Bruggen *et al., J. Biol. Chem.* 196, 389 (1952).
3. D. Steinberg and D. S. Fredrickson, *Proc. Soc. Exptl. Biol. Med.* 90, 232 (1955); H. H. Hernandez *et al., Proc. Soc. Exptl. Biol. Med.* 83, 498 (1953).
4. This work was supported in part by research grants from the American Heart Association and the National Institutes of Health (H-1897 and H-2746). A paper describing our more extensive findings is in preparation.
5. A procedure for a refined gravimetric sterol method was kindly furnished by W. M. Sperry.

4 March 1957

Histochemical Demonstration of Reactivation of Acetylcholinesterase in vivo

Pyridine-2-aldoxime methiodide (2-PAM) and certain other oxime and hydroxamic acid derivatives can reactivate acetylcholinesterase *in vitro* following its inactivation by diisopropylfluorophosphate (DFP) and other irreversible alkylphosphate anticholinesterase agents (*1, 2*). The oximes can also protect animals against the lethal effects of the alkylphosphate anticholinesterases and reverse their neuromuscular blocking action (*3–6*). Although reported findings suggest that the mechanism of the protective action *in vivo* is dependent at least in part on reactivation of acetylcholinesterase, evidence for this has been found only with diaphragmatic muscle (*7*). An alternative explanation is that the protective effect of 2-PAM and similar agents may be due largely to direct combination between the oxime and the anticholinesterase agent in the body before the latter has reacted with the enzyme (*2, 8*), since in most studies the oxime has been injected intraperitoneally prior to or shortly after subcutaneous injection of the anticholinesterase agent (*3, 5*).

Recent work has indicated that the total acetylcholinesterase of neurons may consist of "functional" acetylcholinesterase, external to a lipoid-like membrane, and internal or "reserve" acetylcholinesterase; the latter may represent more recently synthesized enzyme which is stored within the endoplasmic reticulum (*9*). The pharmacological actions of anticholinesterase agents are probably due to inactivation of the former (*10*). If this represents only a small portion of the total acetylcholinesterase, its inactivation and subsequent reactivation might not be detectable by usual homogenate techniques. Furthermore, only limited quantities of quaternary agents, such as 2-PAM, might penetrate the blood-brain barrier and have access to the central nervous system.

In order to determine whether 2-PAM can reactivate acetylcholinesterase of nervous tissue and skeletal muscle *in vivo,* the following study was undertaken (*11*). Cats were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneally) and given atropine sulfate (3 mg/kg, intraperitoneally) and 20 μmole (3.68 mg) of diisopropylfluorophosphate per kilogram, (intravenously) (freshly diluted from a 0.1*M* stock solution in anhydrous propylene glycol). Four cats served as diisopropylfluorophosphate controls. Six received intravenous injections of 2-PAM at the doses and time intervals indicated in Table 1. After the cats had been sacrificed by intravenous administration of air, the stellate and ciliary ganglia and a portion of intercostal muscle were removed and sectioned immediately at 10 μ (ganglia) or 20 μ (muscle) on the freezing microtome. Sections were placed on slides and incubated in acetylthiocholine medium for 5, 10, 20, 40, and 80 minutes, after which they were developed with $(NH_4)_2S$, gold-toned, dehydrated, and mounted, (*12*). Staining was compared

Table 1. Schedule of intravenous administration of 2-PAM and sacrifice of anesthetized cats following intravenous administration of diisopropylfluorophosphate (DFP) (20 μmole/kg).

Cat No.	2-PAM (μmole/kg)	Time of administration after DFP (min)	Time of sacrifice after DFP (min)
1			35
2			35
3			78
4			79
5	20	5	35
6	40	5	35
7	20	20	35
8	40	20	35
9	40	63	78
10	40	64	79

with that of similarly treated control sections from cats which had received no diisopropylfluorophosphate or 2-PAM. Typical results are shown in the photomicrographs in Fig. 1.

In ganglia from control animals, staining for acetylcholinesterase is intense in the cell membranes and cytoplasm of the cholinergic neurons (ciliary ganglion) and considerably lighter in adrenergic neurons (majority in the stellate ganglion). Heavy staining for the enzyme is also seen in the terminations of the cholinergic preganglionic fibers, which are represented in the stellate ganglion chiefly by the protoplasmic tracts. Faint staining for nonspecific cholinesterase can be seen in the glial cells of both ganglia. In the intercostal muscle, acetylcholinesterase activity is concentrated in the subneural apparatus of the motor endplates.

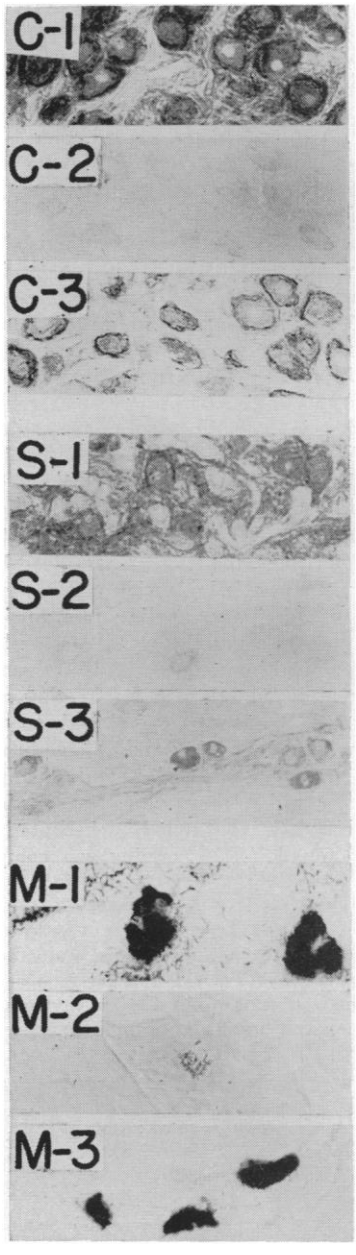

Fig. 1. Sections of ciliary ganglion (*C*), stellate ganglion (*S*) and intercostal motor endplates (*M*) of cats, stained for acetylcholinesterase. 1, Control; 2, diisopropylfluorophosphate (20 μmole/kg) 79 min prior to sacrifice; 3, diisopropylfluorophosphate 79 min and 2-PAM (40 μmole/kg) 15 min prior to sacrifice. Ganglia were incubated 80 min, magnification × 120; endplates were incubated 20 min, magnification × 350.

The acetylcholinesterase of the ganglia of the diisopropylfluorophosphate controls sacrificed at 35 minutes was practically completely inactivated. In the two controls sacrificed at approximately 80 minutes, there was faint staining of the cytoplasm of the cholinergic neurons, suggesting the synthesis of new acetylcholinesterase. This is in keeping with the aforementioned conclusion that synthesis of the enzyme may occur within the endoplasmic reticulum (*9*). Marked reduction occurred in the acetylcholinesterase activity of the motor endplates, which showed, however, considerable variation in intensity.

Findings in all six cats which received diisopropylfluorophosphate followed by 2-PAM were practically identical, despite the differences in doses and time intervals. The cholinergic neurons of the ciliary ganglia showed distinct peripheral staining, and faint but definite staining within the cytoplasm. The same was true of the cholinergic neurons of the stellate ganglion; in this tissue, staining could be seen also in the protoplasmic tracts. Staining of the motor endplates varied, but the average intensity was considerably greater than that of the controls.

When diisopropylfluorophosphate is injected intravenously, most of the agent probably reacts with esterases and other constituents of the tissues within a few minutes; after an hour, it is highly unlikely that any uncombined compound is present (*13*). Therefore, these results appear to demonstrate conclusively that 2-PAM can reactivate acetylcholinesterase of neurons and motor endplates *in vivo* after its inactivation by diisopropylfluorophosphate.

GEORGE B. KOELLE
Department of Physiology and Pharmacology, Graduate School of Medicine, University of Pennsylvania, Philadelphia

References and Notes

1. I. B. Wilson and E. K. Meislich, *J. Am. Chem. Soc.* 75, 4628 (1953); I. B. Wilson, S. Ginsburg, E. K. Meislich, *ibid.* 77, 4286 (1955); I. B. Wilson and S. Ginsburg, *Biochim. et Biophys. Acta* 18, 168 (1955); A. F. Childs *et al., Brit. J. Pharmacol.* 10, 462 (1955).
2. B. J. Jandorf, E. A. Crowell, A. P. Levin, *Federation Proc.* 14, 231 (1955).
3. H. Kewitz, I. B. Wilson, D. Nachmansohn, *Arch. Biochem. and Biophys.* 64, 456 (1956).
4. R. Holmes and E. L. Robins, *Brit. J. Pharmacol.* 10, 490 (1955).
5. B. M. Askew, *ibid.* 11, 417 (1956).
6. J. H. Wills *et al., Science* 125, 743 (1957).
7. H. Kewitz, *Arch. Biochem. and Biophys.* 66, 263 (1957); H. Kewitz and D. Nachmansohn, *ibid.* 66, 271 (1957).
8. J. Wagner-Jauregg and B. E. Hackley, Jr., *J. Am. Chem. Soc.* 75, 2125 (1953); B. E. Hackley *et al., ibid.* 77, 3651 (1955).
9. G. B. Koelle and E. W. Steiner, *J. Pharmacol. Exptl. Therap.* 118, 420 (1956).
10. G. B. Koelle, *ibid.*, in press.
11. This investigation was supported by research grant B-282 (C4) from the National Institute of Neurological Diseases and Blindness, National Institutes of Health, U.S. Public Health Service. Pyridine-2-aldoxime methiodide was kindly supplied by J. Henry Wills, and diisopropylfluorophosphate by William C. Summerson, Army Chemical Center, Md. Photomicrographs were taken by E. W. Glifort. The valuable technical assistance of Priscilla Smart is gratefully acknowledged.
12. G. B. Koelle, *J. Pharmacol. Exptl. Therap.* 103, 153 (1951).
13. B. J. Jandorf and P. D. McNamara, *ibid.* 98, 77 (1950).

1 March 1957

New Mutation in Polioviruses

During the last several years our knowledge of the mutation of polioviruses has been much increased through studies in various *in vitro* systems. Mutants of reduced virulence for monkeys have been discovered (*1*). With regards the size of plaque on monolayers of monkey kidney cells, Dulbecco and Vogt (*2*) have obtained an *r* (rapid) mutant of Brunhilde, and Dubes (*3*) has isolated slow Mahoney, a genetic variant of Mahoney that produces relatively tiny plaques. Dubes (*4*) has also reported on the cold-adapted variants of polioviruses. Heat-resistant variants of polioviruses were also isolated (*5*).

It has been shown (*6*) that spontaneously occurring neutralizing substances for three types of poliovirus can be found in normal adult bovine serums. Although the data thus far accumulated indicate apparently the possible identity of the neutralizing substances with the antibody, it must be admitted that the evidences are not yet conclusive enough, and further experimental work is required before this can be accepted as proved. Accordingly, in this report we refer to these substances as normal bovine serum (NBS) inhibitors for the sake of brevity.

In the work reported here (*7*), mutants of the poliovirus strains, Mahoney and MEF-1, "resistant" to NBS inhibitors, have been obtained readily through serial passages of the parental viruses in HeLa cell cultures with a medium containing 20 to 40 percent inhibitory normal bovine serum. Our strain of HeLa cells has been propagated quite satisfactorily during the last 15 months in medium YLA (Earle, 0.5 percent lactalbumin hydrolyzate and 0.1 percent Difco yeast extract) (*8*), supplemented with only 20 percent normal bovine serum.

A simple method was devised that can