TABLE I.—INTENSITY OF PHYSICAL ACTIVITY AND PROCAINE PENICILLIN G ABSORPTION DATA

r.p.m.[a]	T_e[b] (hr.)	T_r[c] (hr.)	Distance Traveled, M.	$(\bar{R}/\bar{A})_e$[d] (± 95% Confidence Limits) × 10^4 Gm./hr./cm.^{2}	p[e]
0 (12)	0	6.27	...	1.93[d] (0.29)	...
1.04 (6)	3.79	3.23	267	2.18 (0.00)	$0.20 < p < 0.25$
2.05 (8)	3.19	3.15	443	1.97 (0.00)	$0.8 < p < 0.9$
2.81 (12)	2.98	3.08	567	2.34 (0.04)	$0.001 < p < 0.005$
3.83 (7)	2.00	3.26	519	2.57 (0.03)	$0.001 < p < 0.005$

[a] Weighted mean revolutions of activity cage per minute. Number of animals is in parentheses. [b] Mean time for exercise in activity case. [c] Mean time an implanted animal was "resting" or outside the activity cage. [d] Mean absorption rate per mean area attributed to the exercise period in the activity cage as calculated by Eq. 1. The value for zero r.p.m., included for comparison purposes, is actually $(\bar{R}/A)_r$. The 95% confidence limits appear in parentheses. [e] The p value from the 2-tail t test when comparing the mean absorption rate per mean area for zero r.p.m. with rates at another r.p.m. The values at 1.04 and 2.05 r.p.m. should be considered as not significant.

RESULTS AND DISCUSSION

The results of this experiment summarized in Table I showed that when the animals' activity was substantially increased over normal values there was also a significant increase in the magnitude of the mean disk absorption rate per mean area. The present findings tend to substantiate the unverified suggestion made by Kearns (4) that the absorption rate of an implanted pellet (a steroid) could be increased if the patient would massage daily the skin area over the drug.

The results also show that the degree of dispersion about the mean values of $\bar{R}/\bar{A}$ (as reflected by the 95% confidence limits) is much broader for the animals "resting" than it is for those moving at a constant velocity on the activity wheel. The broader dispersion seen in the mean values for the "resting" animals might be expected because there was a wide variation in the intensity of body movement brought on by the use of ether as the anesthetic.

Some clinicians (5, 6) have studied the correlation between physical activity and the magnitude and duration of penicillin serum levels following an intramuscular injection of procaine or benzathine penicillin G. Elevations in penicillin serum levels observed by these investigators after vigorous exercise could be explained by the results of this experiment. If human physical activity increased the dissolution rate of the depot penicillin salt crystals, then elevations in the drug serum level should logically follow, assuming that drug elimination mechanisms remained unchanged before and after exercise.

REFERENCES

(1) Ballard, B. E., and Nelson, E., "Remington's Pharmaceutical Sciences," 13th ed., Mack Publishing Co., Easton, Pa., 1965, pp. 612–640.
(2) Ballard, B. E., and Nelson, E., *J. Pharmacol. Exptl. Therap.*, **135**, 120(1962).
(3) Ballard, B. E., and Nelson, E., *Am. J. Vet. Res.*, **23**, 687(1962).
(4) Kearns, W. M., *J. Urol.*, **47**, 587(1942).
(5) Savolainen, T., and Tommila, V., *Ann. Med. Exptl. Fenn.*, **33**, 345(1955).
(6) Lukash, W. M., and Frank, P. F., *Am. J. Med. Sci.*, **246**, 429(1963).

Cholinesterase Activity and Sulfonamide Absorption in Rats

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Rats administered, orally, a combination of neostigmine methylsulfate and sulfonamide showed an increased 4-hr. blood sulfonamide level as compared to animals administered only sulfonamide. Blood cholinesterase activity, 4 hr. after treatment, was determined and compared with that of control animals.

OVER A half-century ago Barbour and Abel (1) found that physostigmine increased the rate of diffusion of acid fuchsin in frogs. Lewis (2), in 1916, reported the same to be true for trypan red in dogs. Twenty-two years later Cole and Curtis (3) showed that acetylcholine increased the permeability of the marine organism *Nitella*. In the last decade there have been reports of the potentiation of the action of morphine in cats (4), streptomycin in rats (5), and barbital in mice (6) by pretreating with cholinesterase inhibitors. Other reported changes in drug activity brought about by anticholinesterases include the potentiation of the anesthetic activity of a given dose of phenobarbital and pentothal (7) and a lengthening of the duration of the anesthesia.

The increased permeability of *Nitella* in the presence of acetylcholine and the like phenomena seen in *P. vulgarus*, *S. typhosa*, and *P. aeruginosa* with

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TABLE I.—RAT BLOOD CHOLINESTERASE ACTIVITY[a]

Group	Animals, No.	Units of Activity[b]	S.D.	% Depression
Controls	20	0.61	0.07	...
Neostigmine methylsulfate	20	0.49	0.04	20

[a] Neostigmine dose 6 mcg./Kg. [b] Mean value for 20 animals.

Table II.—4-hr. BLOOD SULFONAMIDE[a] LEVELS IN RATS

Group	Animals, No.	mcg./ml.	S.E.
Sulfacetamide	16	1.29	0.1
Sulfacetamide Neostigmine methylsulfate[b]	14	2.83	0.38
Sulfanilamide	16	1.45	0.12
Sulfanilamide Neostigmine methylsulfate	14	4.10	0.37
Sulfaguanidine	16	6.38	0.08
Sulfaguanidine Neostigmine methylsulfate	12	7.35	0.23

[a] All sulfonamides given orally 400 mg./Kg. [b] Neostigmine methylsulfate given orally 6 mcg./Kg.

eserine (8) would indicate a possible relationship between cholinesterase or acetylcholine and membrane permeability in these species.

Cholinesterase and acetylcholine are found throughout the body of higher animals. Alteration of either could affect the tissue permeability which would influence drug absorption. Neostigmine methylsulfate-induced increases of absorption of sulfisoxazole from everted sacs of guinea pig ileum (9) are indicative of the above.

In this work the *in vivo* absorption of sulfonamides in the presence of an anticholinesterase was studied and a comparison made with the sulfonamide absorption in animals with sulfonamide alone. Blood cholinesterase activity was determined 4 hr. after treatment to ascertain the anticholinesterase activity of the neostigmine methylsulfate.

EXPERIMENTAL

Cholinesterase Depression.—In order to correlate any change in sulfonamide absorption with the depression of the esterase, the activity of the enzyme was determined in untreated rats and rats receiving 6 mcg./Kg. of neostigmine methylsulfate 4 hr. prior to withdrawal of blood sample. Blood was removed by cardiac puncture; the enzyme determination was made immediately and expressed in units.[1] The depression was determined to be approximately 20% as shown in Table I.

Sulfonamide Determination.—Sulfacetamide, sulfanilamide, and sulfaguanidine were administered, 400 mg./Kg. orally, to randomly sexed Sprague-Dawley rats weighing 225–275 Gm. Twenty animals were used in each group. A similar number of animals was used for the same sulfonamides administered at the same dose but in combination with 6 mcg./Kg. of neostigmine methylsulfate.

Four hours after the administration of the sulfonamide, 2 ml. of blood to be used for the total sulfonamide determination was removed by cardiac puncture. Samples that clotted before being mixed with the buffer or that may have been diluted with tissue fluid were discarded.

The method used to determine the sulfonamide concentration was the colorimetric method for measuring free and total sulfonamides in tissue used by Bratton and Marshall (10).

Neostigmine methylsulfate increased the blood levels with all three sulfonamides. (Table II.)

DISCUSSION

From the data obtained in this study, it appears that neostigmine methylsulfate increased the absorption of the sulfonamides from the gastrointestinal tract. The observed increase in blood sulfonamide is in agreement with that reported earlier for sulfisoxazole in guinea pigs (9).

It appears probable that the inhibition of cholinesterase, by some mechanism, alters the permeability of the intestinal tract to allow for greater absorption of the drug. It is possible that the anticholinesterase by some combination with the enzyme as a part of cell membrane may change the characteristics of the membrane to allow for greater permeability. Furthermore, it may be that the acetylcholine accumulates in sufficient quantities to change the permeability as it does in *Nitella*. If vasodilation due to the accumulation of acetylcholine is the basic factor in the increased permeability, it should be blocked by atropine. The failure of atropine to block this phenomenon has been shown earlier by Green (7). It appears that cholinesterase–inhibitor combination is the most likely explanation for the observed increases in absorption.

SUMMARY

In this study, rats receiving neostigmine–sulfonamide combinations, orally, showed higher blood levels of the sulfonamides than animals receiving sulfonamide alone. The neostigmine methylsulfate-treated animals showed a 20% depression of blood cholinesterase activity.

Numerous drug agents have anticholinesterase activity. If esterase activity affects absorption, increased absorption of the inhibitor-drug or other drugs used in combination could be a factor in creating undesirable responses. The investigators feel that these findings warrant further investigation.

REFERENCES

(1) Barbour, H. G., and Abel, J. J., *J. Pharmacol. Exptl. Therap.*, **2**, 167(1910).
(2) Lewis, P. A., *J. Expll. Med.*, **23**, 669(1916).
(3) Cole, L. S., and Curtis, J. J., *J. Gen. Physiol.*, **24**, 591 (1938).
(4) Slaughter, D., *J. Pharmacol. Exptl. Therap.*, **68**, 98 (1940).
(5) Green, V. A., *J. Pharm. Sci.*, **52**, 227(1963).
(6) Greig, M. E., and Mayberry, T. C., *J. Pharmacol. Exptl. Therap.*, **102**, 1(1951).
(7) Green, V. A., *J. Pharm. Sci.*, **53**, 762(1964).
(8) *Ibid.*, **51**, 467(1962).
(9) *Ibid.*, **54**, 314(1965).
(10) Bratton, A. C., and Marshall, E. K., *J. Biol. Chem.*, **128**, 537(1939).

[1] A unit of cholinesterase, as used in this study, is defined as the number of milliliters of 0.1 *M* acetic acid derived from 3 ml. of 0.1 *M* acetylcholine bromide solution by the cholinesterase activity in 1 ml. of blood at pH 8.