additive effect of bile shows that this cannot be the entire explanation. Similar increases in activity produced by added taurocholate were reported by Goldman, *et al.* (8), who employed emulsified fats of extremely small droplet size as substrate—and more recently, by Constantin and co-workers (9), utilizing an emulsified olive oil substrate and a pancreatic lipase claimed to be homogeneous.

In the present study both ox and hog bile proved to be excellent activators of lipolysis, in spite of their distinct chemical make-up. Ox bile consists primarily of cholic and desoxycholic acids, with conjugation divided about equally between glycine and taurine; hog bile contains chiefly hyodesoxycholic and hyocholic acids, with exclusive glycine conjugation (1, 2). The dihydroxy acids, desoxycholic and hyodesoxycholic, had the greatest potentiating activity, with the trihydroxy acid, cholic, somewhat less. Dehydrocholic acid, lacking hydroxyl groups, did not show an effect *in vitro*, although *in vivo* it would be expected to exert a pronounced activating effect mediated by bile acids made available through its potent choleretic action (1). The foregoing structural specificity hints at an actual involvement of bile acids in lipolysis by some yet undescribed mechanism.

SUMMARY

Crude and purified bile derivatives showed activating properties on the course of pancreatic lipolysis *in vitro* when employing assay methods utilizing both emulsified and nonemulsified olive oil substrates. Of the salts of purified bile acids tested, desoxycholate was the most potent—a sixfold increase in lipolysis was achieved. Considerable activation was also obtained with hyodesoxycholate, cholate, and taurocholate and with ox and hog biles. In contrast, dehydrocholate did not have *in vitro* effect.

ADDENDUM

Recently an article appeared [Fritz, P. J., and Melius, P., *Can. J. Biochem. Physiol.*, **41**, 719(1963)], which advanced the theory that bile salts activate lipolysis by splitting the enzyme-diglyceride complex, thus increasing the action of lipase on the triglyceride ester. The activating effect was thought *not* to be due to simple emulsification. Glyco and taurocholate were superior to desoxycholate in contrast to the present findings and those of others (1, 15).

REFERENCES

(1) Sobotka, H., "Physiological Chemistry of the Bile," The Williams and Wilkins Co., Baltimore, Md., 1937.
(2) Haslewood, G. A. D., *Physiol. Rev.*, **35**, 178(1955).
(3) Marchis-Mouren, G., Sarda, L., and Desnuelle, P., *Arch. Biochem. Biophys.*, **83**, 309(1959).
(4) Tietz, N. W., Borden, T., and Stepleton, J. D., *Am. J. Clin. Pathol.*, **31**, 148(1959).
(5) Lazo-Wasem, E. A., THIS JOURNAL, **50**, 999(1961).
(6) Sammons, H. G., Frazer, A. C., and Thompson, M., *J. Clin. Pathol.*, **9**, 379(1956).
(7) Minard, F. N., *J. Biol. Chem.*, **200**, 657(1953).
(8) Goldman, M. L., Burton, T. H., and Rayman, M. M., *Food Res.*, **19**, 503(1954).
(9) Constantin, M. J., Pasero, L., and Desnuelle, P., *Biochim. Biophys. Acta*, **43**, 103(1960).
(10) Borgstrom, B., *ibid.*, **13**, 149(1953).
(11) Willstatter, R., Waldschmidt-Leitz, E., and Memmen, F., *Z. Physiol. Chem.*, **126**, 93(1923).
(12) Bier, M., in Colowick, S. P., and Kaplan, N. O., editors, "Methods of Enzymology," Vol. I, Academic Press, Inc., New York, N. Y., 1955, p. 627.
(13) Wills, E. D., *Biochem. J.*, **60**, 529(1955).
(14) Desnuelle, P., *Advan. Enzymol.*, **23**, 129(1961).
(15) De Moerloose, P., *Pure Appl. Chem.*, **3**, 433(1961).

Ceric Sulfate as Permanganate Replacement in Pharmacopeial Assays

By M. M. TUCKERMAN, N. DOCTOFSKY, R. PRICE, R. RAKE, S. RALPH, S. SHENK, and B. A. WISHNEV

Standard 0.1 *N* potassium permanganate was replaced by standard 0.1 *N* ceric sulfate and orthophenanthrolene T. S. indicator in the official assay of ferrous sulfate U.S.P., hydrogen peroxide U.S.P., and sodium perborate N.F. Statistical analysis of the results using the *t* test shows no sufficient reason to doubt that the two methods yield the same mean value at the 10 per cent significance level.

ONE OF THE tests of a rational philosophy is that it contain no unnecessary axioms. One of the tests of a rational system of quantitative analysis might well be that it contain no unnecessary reagents. The "United States Pharmacopeia" and "National Formulary" do not meet this criterion.

This paper focuses attention on the use of strong oxidizing agents as standard solutions in titrimetry. Experience has shown the utility of three agents: ceric ion, dichromate, and permanganate. Of these, dichromate has slowly lost its status, being used now only as a precipitating agent in the determination of quinacrine. With the change in assay of most of the calcium preparations from oxidation of the precipitated oxalate with standard permanganate to a complexometric titration, the need for a standard permanganate solution has decreased. Materials requiring standard permanganate solution may be divided into five classes: (*a*) ferrous sulfate U.S.P., exsiccated ferrous sulfate U.S.P., hydrogen peroxide solution U.S.P., and sodium perborate N.F.; these are titrated directly. (*b*) Dibasic calcium phosphate U.S.P. and cherry juice U.S.P.; these require titration of precipitated calcium oxalate. (*c*) Lead monoxide N.F., lead subacetate solution N.F., and diluted lead subacetate solution N.F.; these require titration of oxalic acid left in the filtrate after precipitation of lead oxalate. (*d*) Sodium nitrite U.S.P. which requires a residual titration involving both standard permanganate and standard oxalic acid.

Received June 3, 1963, from the Department of Chemistry, School of Pharmacy, Temple University, Philadelphia, Pa.
Accepted for publication August 7, 1963.
The authors extend their thanks to Dr. Edward Fackenthal, Department of Physics, School of Pharmacy, Temple University, for the statistical analysis of the collected data.
Presented to the Scientific Section, A.PH.A., Miami Beach meeting, May 1963.

TABLE I.—COMPARISON OF RESULTS FOR FERROUS SULFATE U.S.P.

Analyst	Permanganate Trial A	Permanganate Trial B	Permanganate Trial C	Ceric Sulfate Trial A	Ceric Sulfate Trial B	Ceric Sulfate Trial C
1	100.5	100.5	100.3	99.0	98.3	98.9
2	100.6	100.2	100.3	99.1	99.3	99.0
3	99.8	99.8	99.8	102.8	102.8	102.8
4	100.1	100.4	100.3	99.9	99.8	100.0
5	100.2	99.9	100.4	99.1	99.8	99.4
6	102.6	101.6	101.5	102.9	102.7	102.5
Av. ($\overline{X}$)			100.49			100.46
S.D. (σ)			1.87			4.46
sp				1.327		
t				0.1356		
For $\alpha = 0.10$				$t > \lvert 1.69 \rvert$		

TABLE II.—COMPARISON OF RESULTS FOR HYDROGEN PEROXIDE SOLUTION U.S.P.

Analyst	Permanganate Trial A	Permanganate Trial B	Permanganate Trial C	Ceric Sulfate Trial A	Ceric Sulfate Trial B	Ceric Sulfate Trial C
1	2.32	2.30	2.34	2.50	2.51	2.51
2	2.18	2.22	2.34	2.18	2.23	2.36
3	2.94	2.93	2.96	2.95	2.96	2.93
4	2.40	2.31	2.30	2.48	2.36	2.34
5	2.93	2.95	2.95	2.90	2.93	2.88
Av. ($\overline{X}$)			2.551			2.604
S.D. (σ)			0.548			0.470
sp				0.3104		
t				0.3543		
For $\alpha = 0.10$				$t > \lvert 1.70 \rvert$		

TABLE III.—COMPARISON OF RESULTS FOR SODIUM PERBORATE N.F.

Analyst	Permanganate Trial A	Permanganate Trial B	Permanganate Trial C	Ceric Sulfate Trial A	Ceric Sulfate Trial B	Ceric Sulfate Trial C
1	9.60	9.55	9.47	9.51	9.60	9.61
2	9.60	9.51	9.48	9.48	9.36	9.60
3	9.56	9.55	9.47	9.81	9.76	9.79
4	9.83	9.80	9.72	9.69	9.72	9.78
5	9.65	9.58	9.37	9.68	9.64	9.56
6	9.39	9.44	9.31	10.33	10.30	10.29
Av. ($\overline{X}$)			9.549			9.751
S.D. (σ)			0.230			0.461
sp				2.215		
t				0.2736		
For $\alpha = 0.10$				$t > \lvert 1.69 \rvert$		

(*e*) Titanium dioxide N.F., potassium permanganate U.S.P., and potassium permanganate tablets N.F.

The purpose of this paper is to compare the results of titrations using permanganate and ceric sulfate for substances in the first group. Other methods of assay will be proposed at a later time for the other groups.

METHOD

The assays reported in the tables were performed by senior pharmacy students who had taken 1 year of quantitative analysis, during which time they had experience with both permanganate and ceric ion. All assays were carried out by the official methods of the "United States Pharmacopeia" (1) or "National Formulary" (2) and again carried out substituting standard 0.1 *N* ceric sulfate solution (1) and orthophenanthrolene T.S. (1) for the permanganate. No other changes were made in the procedure. The samples used were a single solid sample of ferrous sulfate U.S.P., a single solid sample of sodium perborate N.F., and a previously unopened bottle of commercially prepared hydrogen peroxide solution U.S.P. containing 0.05% acetanilid as a preservative.

RESULTS AND DISCUSSION

The results obtained for ferrous sulfate U.S.P. are listed in Table I, those for hydrogen peroxide solution U.S.P. in Table II, and those for sodium perborate N.F. in Table III. The results, viewed as a whole, show that although there is generally good agreement between the three values obtained by any one analyst, there is considerable variation between analysts. This variation is shown by the values of the standard deviations. Although these may seem excessively large to one who is accustomed to comparing only the results of his own three repetitive analyses, they are actually in agreement with other studies of laboratory and analyst variability (3).

The results for hydrogen peroxide solution U.S.P. are a cause for concern because of the large standard deviation. The cause for the deviation has not been investigated, but it may, in part, be caused by the small (2-ml.) sample size, since the accuracy of measurement of a sample containing oxygen bubbles is questionable, and decomposition of the sample catalyzed by traces of heavy metals adsorbed on the walls of the glass equipment used, is possible.

This study again emphasizes the danger in placing confidence in a single assay, even when done by a trained person using a well established technique. It also shows the danger of placing confidence in replicate assays performed in parallel by the same analyst, even when the standard deviation for the replicates appears acceptable.

CONCLUSIONS

As shown by the statistical data included in the tables, there is no reason to think that the replacement of permanganate with ceric sulfate changes the mean value of the assay results. Although smaller values for the standard deviations might have been obtained by rejecting some of the results, all data collected have been reported.

REFERENCES

(1) "United States Pharmacopeia," 16th rev., Mack Publishing Co., Easton, Pa., 1960.
(2) "The National Formulary," 11th ed., J. B. Lippincott Co., Philadelphia, Pa., 1960.
(3) *Anal. Chem.*, **23**, 1528(1951).

Vasopressin Tachyphylaxis

By P. N. PATIL, A. TYE, and J. W. NELSON

Pretreatment with D-*levo*- ephedrine is known to augment the pressor effect of vasopressin in dogs. It also delayed the development of vasopressin tachyphylaxis in this study. Methoxamine did not produce the same effects. Reserpine augmented the pressor response and arrested the tachyphylaxis. Increasing doses of vasopressin then produced increases in pressor response, an effect which suggests a possible bioassay for vasopressin. No cross tachyphylaxis to pressor effects were observed between vasopressin and angiotensin.

GEILING AND CAMPBELL (1) and Jones and Schlapp (2) have noted the decrease in pressor response to successive doses of posterior pituitary extract. Hogben, *et al.* (3), studied the development of tolerance to posterior pituitary lobe extract in the spinal cat and concluded that it is a function of dose and time interval between doses. By spacing repeated doses at appropriate time intervals, they were able to obtain pressor responses of the same character and magnitude as the one produced by the first injection. Woodbury and Wilks (4) found that in ouabain-treated animals tachyphylaxis to vasopressin developed less readily than in control animals; they suggested that vasopressin tachyphylaxis is a "pseudo-tachyphylaxis." Gardier and Abreu (5) showed that tolerance to vasopressin can be prevented by bilateral carotid sinus denervation and midcervical vagotomy. We investigated the effects of pretreatment with D-*levo*-ephedrine, methoxamine, and reserpine in this study. The sympathomimetic amine, D-*levo*-ephedrine, is a good antagonist of the coronary constriction produced by vasopressin (6); methoxamine is a sympathomimetic amine that produces peripheral effects without cardiotonic effects (7, 8); reserpine blocks carotid and vagal reflexes (9, 10). Cross tachyphylaxis between angiotensin and vasopressin and the effects of renin were also studied.

EXPERIMENTAL

Twenty-five mongrel dogs of each sex, weighing from 7 to 11 Kg., were anesthetized with 35 mg./Kg. of pentobarbital i.p. Both vago-sympathetic nerves were severed. With the usual hemodynamic setup, blood pressure was recorded from the right carotid artery on a kymograph. The trachea was always cannulated. All drugs were dissolved in physiological saline and injected into the femoral vein *via* an indwelling catheter.

Received July 16, 1963, from the College of Pharmacy, The Ohio State University, Columbus.
Accepted for publication August 19, 1963.
Presented to the Scientific Section, A.PH.A., Las Vegas meeting, March 1962.

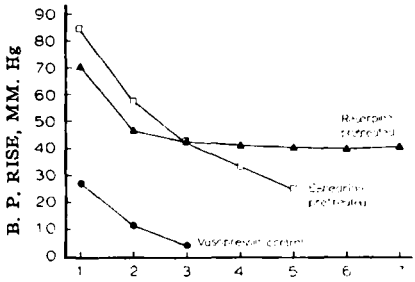

Fig. 1.—The effects of reserpine and D-*levo*-ephedrine pretreatment on vasopressin tachyphylaxis. Each point represents the average blood pressure rise of four to five dogs. The standard errors of the mean varied from 9 to 20% of the average values.

The reserpinized dogs were prepared by administering 0.5 mg./Kg. reserpine i.p. each day for 2 days; the blood pressure was measured as above on the third day. Anesthesia was produced with 15 to 20 mg./Kg. of pentobarbital i.p. in these dogs. Additional pentobarbital was given when necessary to maintain surgical anesthesia.

The following drugs were used: D-*levo*-ephedrine HCl, methoxamine HCl,[1] vasopressin,[2] reserpine phosphate,[3] angiotensin,[4] and hog renin.[5]

RESULTS

In control dogs, vasopressin 0.3 u./Kg. at 30-minute intervals resulted in a progressive reduction of the pressor effect. Tachyphylaxis was practically

[1] Marketed as Vasoxyl by Burroughs Wellcome and Co., Inc., Tuckahoe, N. Y.
[2] Marketed as Pitressin by Parke, Davis and Co., Detroit, Mich.
[3] Marketed as Serpasil by Ciba Pharmaceutical Products, Inc., Summit, N. J.
[4] Marketed as Hypertensin-Ciba by Ciba Pharmaceutical Products, Inc., Summit, N. J. Courtesy of Dr. A. J. Plummer.
[5] Courtesy of Dr. O. M. Helmer, Eli Lilly and Co., Indianapolis, Ind.