3. G. L. WHITBY, J. AXELROD and G. HERTTING, *Nature* **187**, 604 (1960).
4. G. HERTTING, J. AXELROD, G. L. WHITBY and R. PATRICK, *Fed. Proc.* **20**, 167 (1961).
5. A. CARLSSON, E. ROSENGREN, A. BERTLER and J. NILSSON, In *Psychotropic Drugs* p. 363, Elsevier Publishing Co., Amsterdam (1957).
6. J. H. BURN and M. J. RAND, *J. Physiol.* **144**, 314 (1958).
7. M. L. TAINTER and D. K. CHANG, *J. Pharmacol.* **39**, 193 (1927).
8. A. FROEHLICH and O. LOEWI, *Arch. exp. Pharm. u. Path.* **62**, 159 (1910).
9. M. F. LOCKETT and K. E. EAKINS, *J. Pharm. Pharmacol.* **12**, 513 (1960).
10. H. J. SCHUMANN and E. WEIGMANN, *Arch. exp. Pharm. Path.* **240**, 275 (1960).

Effect of a phosphatido-peptide fraction of intestinal tissue on the intestinal absorption of a quaternary ammonium compound*

(*Received* 8 *June* 1961)

PREVIOUS studies have shown that although quaternary ammonium compounds are incompletely absorbed from the intestine, there is always a portion that is absorbed and this portion is absorbed fairly rapidly following administration.[1] It has been postulated that the absorbed portion of the quaternary ammonium compound, which exists as a cation at all physiological levels of pH, is transferred across the gut wall as a neutral complex by virtue of combination with an endogenous anion.[2] Since a number of phosphorus-containing compounds have been implicated as possible carriers in the transport of endogenous inorganic and organic cations across biological membranes,[3, 4] the role of such phosphorus-containing anions in the transport of exogenous organic cations is now under investigation.

A phosphorus-containing fraction, first isolated from brain and designated "phosphatido-peptide" by Folch,[5] has been found in intestine, as well as in heart, liver, lung, spleen, pancreas and kidney.[6] The phosphatido-peptide represents a fifth category of tissue phosphorus compounds, since it cannot be classified as acid-soluble phosphorus, free phosphatides, nucleic acids or phosphoprotein, as usually defined.[7] The phosphatido-peptide fraction is constituted by inositol, phosphate, esterified fatty acids, sphingosine or sphingosine-like material and amino acids combined in polypeptide chains.[5, 6] This report presents preliminary evidence that the phosphatido-peptide fraction from intestinal tissue may be involved in the intestinal absorption of a mono-quaternary ammonium drug, benzomethamine (N-diethylaminoethyl-N′-methylbenzilamide methobromide).

METHODS

The material used in these studies was a relatively crude fraction of rat small intestine, prepared by the method of Huggins and Cohn,[6] including removal of (1) acid-soluble components with trichloroacetic acid, (2) phospholipides with alcohol-ether, (3) nucleic acid with 10% sodium chloride, and (4) extraction of the crude fraction from the remaining phosphoprotein by chloroform-methanol-HCl. This acidified chloroform-methanol extract was evaporated to near dryness *in vacuo* and the oily residue was taken up and suspended in Krebs-Henseleit phosphate buffer, pH 7·4, for use in the absorption studies. This suspension was regarded and used as the phosphatido-peptide fraction.

Non-fasted male rats of the Sprague–Dawley strain, weighing 180–210 g, were used. The degree of intestinal absorption was determined, *in vivo*, using either a single-loop preparation or a double-loop preparation, as previously described.[2] Double-loop preparations were used for controls, the loop positions of the control and experimental solutions to be compared being alternated in successive rats. Three kinds of control solutions were used: (1) benzomethamine dissolved in buffer, as a standard; (2) benzomethamine dissolved in a buffered solution of reagent blank of the extraction procedure, as a negative control; (3) benzomethamine dissolved in a buffered solution of pure albumin carried through the extraction procedure, as a pseudo-tissue control. A constant volume of 0·5 ml/loop

* This work was supported in part by a Research Grant (B-1966) from the Institute of Neurological Diseases and Blindness, U.S. Public Health Service, Bethesda, Maryland.

was used and the dose of benzomethamine was 0·5 mg/loop. All absorption studies were for periods of 3 hr and the entire intestinal loop and contents were used for chemical determination, by previously described methods,[2, 8] of the benzomethamine still present after this time.

Levels of significance were calculated using the *t*-test.[9]

RESULTS

The effect of varying amounts of the phosphatido-peptide fraction on the *in vivo*-absorption of benzomethamine in the rat is shown in Fig. 1. The data were obtained using 6 separate extracts of intestinal tissue. In all cases in which the amount of extract used was equivalent to one or more grams of tissue there was a significant increase in the amount of benzomethamine absorbed, as compared with each of the 3 types of controls ($P < 0{\cdot}01$).* Even more noteworthy is the fact that the degree of increase in absorption of the quaternary compound was a function of the amount of phosphatido-peptide present in the intestinal loop.

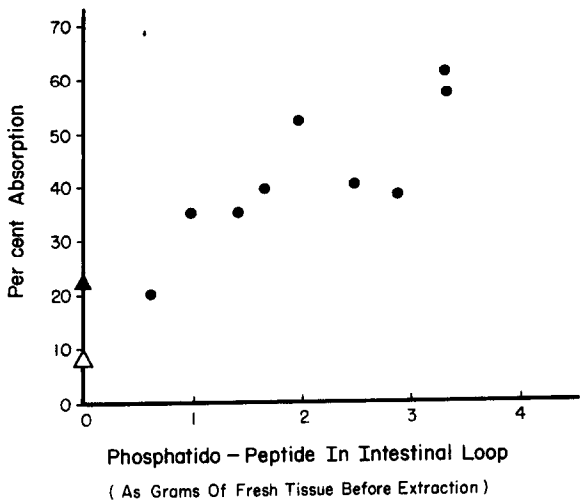

FIG. 1. Effect of phosphatido-peptide on the absorption of benzomethamine in the rat. ▲: Standard and negative controls (mean of 8 determinations). △: Pseudo-tissue controls (mean of 8 determinations). ●: Each point represents the mean per cent absorption in 4 animals in which the loops contained the phosphatido-peptide fraction. Dose of benzomethamine: 0·5 mg/loop.

Other phosphorus-containing compounds were investigated to determine their effect on absorption of the quaternary ion. Simple compounds, such as *o*-phosphorylserine and phosphorylcholine, as well as the phosphatides, phosphatidyl serine, phosphatidyl ethanolamine and *a*-lecithin, were without effect on the degree of absorption of benzomethamine *in vivo*. Inositol-2-monophosphate and inositol phosphatide, which have been shown to be present in the phosphatido-peptide fraction,[6, 7] also had no effect on the absorption of benzomethamine *in vivo*.† It would appear, thus far, that the facilitating effect on the absorption of a quaternary compound is relatively specific for, and dependent on the integrity of, the phosphatido-peptide fraction, although all the constituents of this fraction have not been identified and tested individually here, or elsewhere,[10] for this effect on absorption.

* There was an insignificant difference between the degree of absorption of the standard and negative controls, but the degree of absorption in both these controls was significantly ($P < 0{\cdot}01$) higher than in the pseudo-tissue controls.

† Phosphatidyl serine, phosphatidyl ethanolamine and inositol phosphatide were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio. Inositol-2-monophosphate, α-lecithin, *o*-phosphorylserine and phosphorylcholine were purchased from California Corporation for Biochemical Research, Los Angeles, California.

Preliminary evidence has been obtained, *in vitro*, using dialysis and two-phase solvent partition techniques, for the formation of a complex between the phosphatido-peptide fraction and benzomethamine.

In previous studies it had been shown that intestinal mucus, and polysaccharides extracted from mucus, inhibited the absorption of quaternary ammonium compounds.[1, 2] While the role of phosphatido-peptide in the mechanism of absorption has not been established, this is the *first* evidence that intestinal absorption of a quaternary ammonium compound can be enhanced by material extracted from intestinal tissue.

Department of Pharmacology and Experimental Therapeutics,
Boston University School of Medicine,
Boston, Mass.

RUTH R. LEVINE
ALICE F. SPENCER

REFERENCES

1. R. M. LEVINE, M. R. BLAIR and B. B. CLARK, *J. Pharmacol.* **114**, 78 (1955).
2. R. R. LEVINE and E. W. PELIKAN, *J. Pharmacol.* **131**, 319 (1961).
3. L. E. HOKIN and M. R. HOKIN, *Int. Rev. Neurobiol.* **2**, 99 (1960).
4. A. K. SOLOMON, F. LIONETTI and P. F. CURRAN, *Nature* **178**, 582 (1956).
5. J. FOLCH, In *Phosphorus metabolism* (Edited by W. D. MCELROY and H. B. GLASS) Vol. II, Johns Hopkins Press, Baltimore, p. 186 (1952).
6. C. G. HUGGINS and D. V. COHN, *J. Biol. Chem.* **234**, 257 (1959).
7. F. N. LEBARON and J. FOLCH, *J. Neurochem.* **1**, 101 (1956).
8. R. R. MITCHELL and B. B. CLARK, *Proc. Soc. Exp. Biol. Med.* **81**, 105 (1952).
9. G. W. SNEDECOR, *Statistical Methods* (4th ed.) The Iowa State College Press, Ames, Iowa (1946).
10. R. R. LEVINE, *J. Pharmacol.* **131**, 328 (1961).

Effect of injected estradiol on the uptake of α-amino*iso*butyric acid by tissues of the ovariectomized rat

(*Received* 28 *June* 1961)

ESTROGEN treatment produces an elevation of free amino acids in the rat uterus.[1] The accumulation of an amino acid that is not metabolized to any appreciable extent, α-amino*iso*butyric acid (AIB), is also enhanced by estrogens; thus, a few years ago, Noall and coworkers[2] reported a three-fold increase, on a wet-weight basis, in uterine AIB 20 hr after a single subcutaneous injection of estradiol. This report follows the uptake by the uterus and other tissues of AIB, after estrogen treatment, and extends the previous observations which were limited to one time period.

METHODS

Long–Evans female rats, three weeks of age, were injected intraperitoneally with 1·25 μc (0·25 μmoles) of AIB-1-C^{14}. At intervals of from 0 to 30 hr before sacrifice, groups of 4 rats were anesthetized with ether and given intravenous injections of 0·1 μg of estradiol in 0·1 ml of saline, or of saline alone for controls. The rats were sacrificed under ether anesthesia 20 hr after the injections of AIB, in order that our data could be compared with that of Noall *et al.*[2] Blood was taken from the heart, the uteri were removed and blotted, and portions of muscle, from the hamstring mass, and of liver were obtained.

The 4 blood samples from each group of rats were pooled. After clot formation, an aliquot of serum was mixed with 75% ethanol to precipitate protein; the supernatant fraction was saved for the measurement of radioactivity. The other tissue samples were pooled in tared all-glass homogenizers. They were weighed (to 0·1 mg) before and after drying at 75 °C for 24 hr and then were homogenized in 75% ethanol. The radioactivity in the supernatant fraction was measured in a liquid scintillation counter. Each vial contained 10 ml of dioxane, in which were dissolved 0·9 g of naphthalene, 70 mg of 2:5-diphenyloxazole, 0·5 mg of *p*-bis[2-(5-phenyloxazolyl)]-benzene, and 0·15 ml of the supernatant material.