PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 151, 707-710 (1976)

Evaluation of [^{14}C]Aminopyrine Clearance for Determination of Gastric Mucosal Blood Flow[1] (39290)

LARRY L. TAGUE AND EUGENE D. JACOBSON

Department of Physiology, The University of Texas Medical School at Houston, Houston, Texas 77025

Several markers have been used to determine gastric mucosal blood flow by the clearance technic. These include aminopyrine (1), [^{14}C]aniline (2), and neutral red (3). These dissimilar substances are lipid permeable at neutral pH with pK_a values of 5 to 10, and they are transported irreversibly across the gastric mucosa into acidic luminal contents. Both aminopyrine and aniline are toxic agents for human use, and the analytic procedure for their quantification in body fluids is time consuming. Determination of neutral red requires a sophisticated spectrofluorometer which will accurately discriminate between 540 and 590 nm. Objections to the use of [^{14}C]aniline relate to its toxicity, since the quantity of unlabeled carrier aniline administered is sufficient to produce methemoglobinemia and its clearance is significantly less than that of aminopyrine. Limitations of clearance technics in the stomach have been reviewed extensively in several reports (4–6).

The present study was designed to reduce the assay time (a primary objection to the aminopyrine clearance method as previously described) and evaluate the utility of ^{14}C-labeled aminopyrine alone at very low concentrations for determining mucosal blood flow, thereby, eliminating its toxicity for human subjects.

Methods. Dogs weighing 15–20 kg each were fasted for 24 hr, anesthetized with chloralose and urethane, and subjected to laparotomy to expose the stomach. The gastric fundus was incised and a flap of the wall was mounted in a lucite chamber with its blood supply intact (7). A femoral artery and both femoral veins were cannulated for measurement of systemic arterial blood pressure, infusion of aminopyrine or drug and withdrawal of blood. After an initial 10-ml blood sample was obtained and mixed with heparin sodium (100 units), a bolus of 3 μCi of [^{14}C]aminopyrine (Amersham/Searle, 52.8 μCi/mg) was injected intravenously in 168 ml saline with or without 15 mg/kg unlabeled aminopyrine. Then a continuous infusion was started at a rate of 0.0125 μCi/min [^{14}C]aminopyrine (with or without 0.0625 mg/min unlabeled material). Each animal was allowed to equilibrate with aminoyprine for 1 hr prior to starting the study. At hourly intervals 10-ml blood samples were drawn in heparin and centrifuged at 4° to obtain plasma. Every 15 min a fresh solution (10 ml 0.1 *N* HCl in physiological saline) was placed on the mucosa after removing the previous bathing solution and measuring its volume. All samples were maintained on ice and evaluated the same day or frozen and measured the following day.

Conscious animal studies were conducted using a dog which had been provided with both a gastric fistula and a Heidenhain pouch. Only [^{14}C]aminopyrine was administered in this study in which histamine was employed to stimulate secretion and augment mucosal blood flow, and vasopressin was used to depress both functions (1). Pouch secretions were collected for quantification of ^{14}C clearance.

The rate of infusion of histamine (base) was 1.2 μg/kg-min and of vasopressin was 10 units/hr in both anesthetized and conscious dogs.

Aminopyrine clearance was measured by both radiometric and spectrophotometric methods. For spectrophotometric determinations a modification of the method described by Brodie and Axelrod was employed (8). One milliliter of plasma or gastric bathing medium was pipetted into 50-ml glass-stopped conical test tubes containing 0.5 ml 1 *N* NaOH and 5.0 ml dichloromethane (Matheson, Coleman, and Bell pesti-

[1] Supported by NIH Grant No. 5 ROI AM 15977-04.

cide quality). The aminopyrine was extracted into the organic phase by gentle shaking on a dry Dubnoff incubator shaker at a rate of 100/min. The formation of an emulsion was avoided. After shaking for 15 min the aqueous (top) phase was carefully removed to minimize loss of dichloromethane. The organic phase was washed twice with 2-ml aliquots of a saturated sodium borate solution to remove color and protein. Five milliliters of 0.1 *N* HCl was added, and the aminopyrine was extracted into the aqueous phase by gentle shaking for 15 min. The absorbance of the aqueous (top) phase was read at 260 nm using a spectrophotometer (Gilford Model 240). An appropiate blank (1.0 ml H_2O) and standard (1.0 ml of an 80-μg aminopyrine/ml solution) were carried through the extraction procedure and measured. Aminopyrine recoveries of 95–105% were obtained with this method.

For radiometric evaluation of gastric mucosal clearance 1 ml of gastric bathing medium was pipetted directly into scintillation vials and mixed with 10 ml of PCS (Amersham/Searle). Plasma samples were extracted as described for spectrophotometric evaluation, and 4.0 ml of the final HCl phase was mixed with 15 ml of PCS for counting. Appropriate blanks were also counted. Gastric samples were counted for 10 min and plasma samples for 20 min. After subtraction of blanks, cpm/ml was determined and the following equation used to calculate clearance:

Clearance (ml/min) = [(cpm/ml gastric juice)/(cpm/ml Plasma)] × ml/min gastric juice secreted plus bathing fluid. If gastric juice samples had color contaminants, a quench correction was necessary. Significance of differences was evaluated using the Student's *t* test with an acceptable probability of < 0.05 (9).

Results. No significant differences were found between mucosal blood flows estimated simultaneously by [^{14}C]aminopyrine clearance and by unlabeled aminopyrine in anesthetized dogs during manipulation of the flows with histamine and vasopressin (Fig. 1). Both [^{14}C]aminopyrine and the unlabeled base were combined and administered intravenously; after basal clearance had been measured for an hr, histamine was infused for 1 hr to increase mucosal blood flow, and then histamine and vasopressin were infused for 1 hr to decrease mucosal blood flow. In Fig. 2, the two methods of blood flow estimation are compared in a scattergram and linear regression analysis over a flow range of 1 to 15 ml/min. The coefficient of correlation was 0.87.

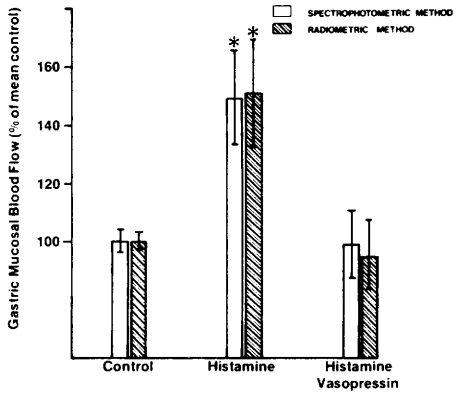

FIG. 1. The effects of histamine and histamine plus vasopressin on gastric mucosal blood flow as measured spectrophotometrically and radiometrically (N = 12). * = significant difference from control values.

The data shown in Fig. 3 demonstrate that [^{14}C]aminopyrine may be used alone without protection against metabolic degradation afforded by unlabeled aminopyrine. The magnitude of mucosal flow changes with histamine and histamine plus vasopressin are the same as shown in Fig. 1 where both labeled and unlabeled aminopyrine were present.

Aminopyrine extraction across the gastric mucosa is neither "carrier" limited nor actively transported (4). A large bolus of unlabeled aminopyrine was injected intravenously during radiometric measurement of histamine stimulated mucosal blood flow with [^{14}C]aminopyrine alone and there was no significant change in mucosal clearance of the labeled marker (Fig. 4).

[^{14}C]Aminopyrine was employed as the sole marker in chronic animal studies, and its clearance was measured across a Heidenhain pouch during histamine-induced augmentation of blood flow and during inhibition of histamine stimulation with vasopressin (Fig. 5). Disappearance of residual ^{14}C-label from the dog was complete within 36 hr. This was determined by monitoring blood levels and excretion.

Discussion. The aminopyrine clearance

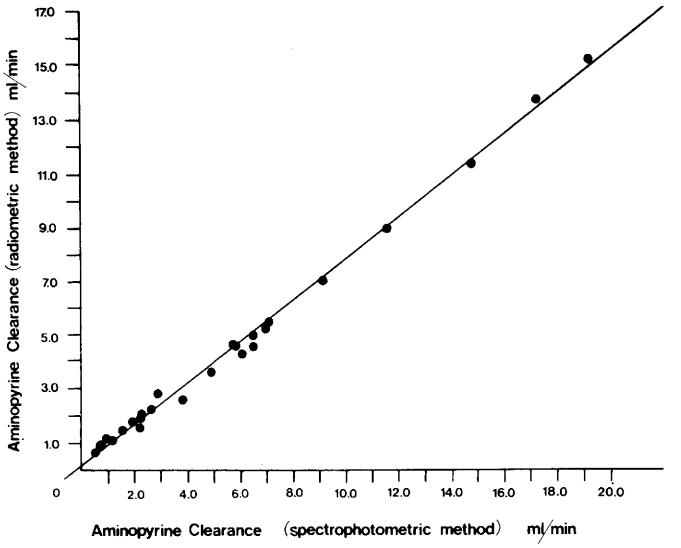

FIG. 2. A linear regression of radiometric clearance vs. spectrophotometric clearance (r = 0.87, b = 0.97, N = 36).

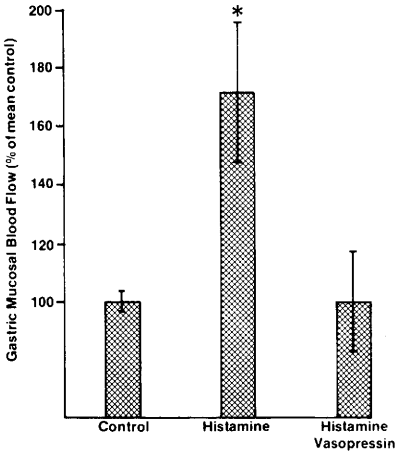

FIG. 3. Measurement of mucosal blood flow changes stimulated by histamine and histamine plus vasopressin with [^{14}C]aminopyrine alone (N = 4). * = significant difference from control values.

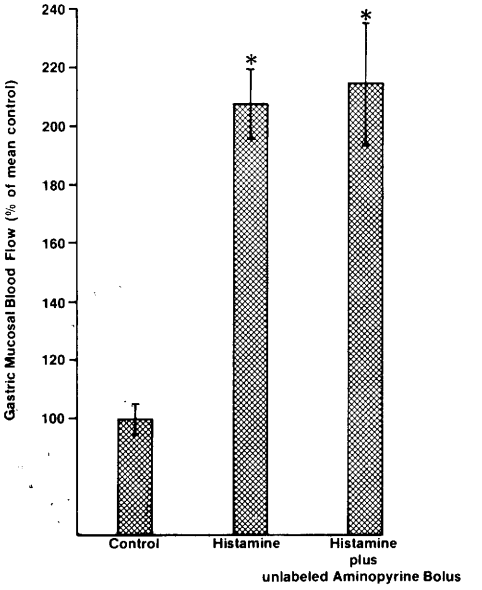

FIG. 4. The effects of an unlabeled aminopyrine bolus on the determination of [^{14}C]aminopyrine clearance during histamine stimulation (N = 4). * = significant difference from control values.

technic for estimation of gastric mucosal blood flow is widely used and is the reference method for other markers in animal studies (2–6). The method has two drawbacks. The extraction and reading of samples spectrophotometrically is time-consuming and limits the total number of samples which can be handled each day. In addition, repeated use of relatively large amounts of unlabeled aminopyrine over a period of months leads to depression of hematopoietic activity and agranulocytosis in chronic animal experiments.

Eliminating the need for extraction of gastric juice samples reduces the number of extractions by 80% and saves an equal proportion of time. Using [^{14}C]aminopyrine alone reduced the total dose of aminopyrine administered during a 4-hr experiment to less than 0.01% of the amount of unlabeled material that would have been necessary to estimate the same number of mucosal blood

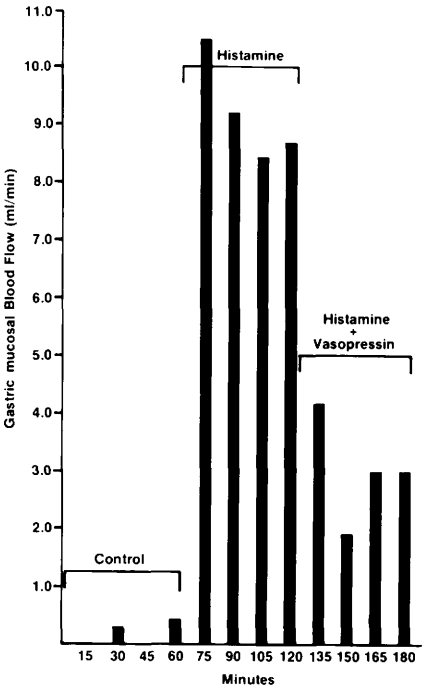

FIG. 5. The effects of histamine and histamine plus vasopressin on Heidenhain pouch clearance of [^{14}C]aminopyrine in a conscious dog.

flows spectrophotometrically. This reduction in total dose should eliminate toxicity and make the marker suitable for human use.

A question raised in the past about the gastric clearance of aminopyrine has been the possibility of active transport limitation within the stomach. This possibility is suggested by observations showing seemingly parallel increases in H^+ ion secretion and aminopyrine clearance (1, 4, 10). Indirect evidence that there is no active transport of aminopyrine includes these findings (4): (i) aminopyrine clearance can be increased with vasodilator drugs without altering secretion, (ii) chemically dissimilar agents are cleared by the stomach as readily as aminopyrine, and (iii) increased concentration of H^+ ion in secreted gastric juice does not cause an increased ratio of juice/plasma concentrations of aminopyrine. In the present study more direct evidence on this question was obtained (Fig. 4). If active transport of [^{14}C]aminopyrine played a significant role in its clearance, the addition of a large unlabeled pool would (by competitive inhibition) have decreased clearance of the labeled aminopyrine. Injection of an unlabeled bolus containing 2000 times more absolute aminopyrine than the total labeled dose did not inhibit clearance of the latter.

Conclusion. This study has demonstrated high correlation between the radiometric and spectrophotometric determinations of gastric mucosal aminopyrine clearance. The radiometric method is technically easier, allows a larger number of samples to be determined and is safer to the subject. The clearance of small amounts of [^{14}C]aminopyrine was unaffected by large doses of unlabeled aminopyrine showing that mucosal extraction is not concentration limited. Small amounts of [^{14}C]aminopyrine may provide an excellent tool for examining the role of mucosal blood flow in the pathogenesis of gastric disease in man.

1. Jacobson, E. D., Linford, R. H., and Grossman, M. I., J. Clin. Invest. **45,** 1 (1966).
2. Curwain, B. P., and Holton, P., Brit. J. Pharmac. **41,** 384P (1971).
3. Aures, D., Guth, P. H. and Grossman, M. I., Gastroenterology, **68,** A-200 (1975).
4. Jacobson, E. D., Gastroenterology **54,** 434 (1968).
5. Koch, H., Acta Hepato-Gastroenterol. **19,** 305 (1972).
6. Lanciault, G., and Jacobson, E. D., Gastroenterology, in press.
7. Mao, C.C., Shanbour, L. L., Hodgins, D. S., and Jacobson, E. D., Gastroenterology **63,** 427 (1972).
8. Brodie, B. B., and Axelrod, J. I., Pharmac. Exp. Ther. **99,** 171 (1950).
9. Snedecor, G. A. and Cochran, W. G., "Statistical Methods," 6th ed., Iowa State Press, Ames (1967).
10. Harper, A. A., Reed, J. D., and Smy, J. R., J. Physiol. (London), **191,** 795 (1968).

Received September 26, 1975. P.S.E.B.M., 1976, Vol. 151.