Effect of Ureteral Stop Flow on PAH in Lumen and Cortex Homogenate in the Rat Kidney[1] (35562)

GASPAR CARRASQUER[2] AND THADDEUS W. WILCZEWSKI[3]

University of Louisville, School of Medicine, Louisville, Kentucky 40202

It is commonly accepted that *p*-aminohippurate (PAH) is secreted in the proximal tubule of the kidney (1). Renal tubule cells like other epithelial cells can actively transport solute through them, from a "source" solution on one side to a "sink" solution on the other side. This may be accomplished by: (i) A "push" system, in which case the "pump" is on the cellular face opposite to the direction in which the substance is moved, *i.e.*, the source side. In this case the intracellular concentrations are generally higher than in the "source" and "sink" solutions; for example, glucose transport by intestinal epithelium (2, 3). (ii) A "pull" system, with the "pump" located in the sink face of the cell. In this case the intracellular concentrations are lower than in the "source" and "sink" solutions; for example, sodium transport by toad bladder (4). The mechanism by which PAH is secreted in mammals has not been well established yet, although various investigators have suggested that the mechanism is that of a "push" system, that is, the active pump is located in the peritubular membrane (5–8). If this hypothesis is true, an electrochemical concentration gradient should exist between cell and interstitium, and between cell and lumen with an electrochemical concentration in the cell higher than in the two other compartments.

Since the movement of tubular fluid carries away the secreted material, the gradients produced by the active transport process will be limited. On the other hand, if the intraluminal movement of fluid is stopped or greatly reduced, the transmembrane gradient should be magnified at the site of active transport, that is, between interstitium and cell only, if the mechanism of PAH secretion is that of a "push" system.

The purpose of the present work was to determine the site of the PAH pump by location of the concentration gradient (peritubular or luminal membrane). Since the movement of fluid along the nephron is practically stopped during ureteral obstruction in mannitol leading (8–10) ureteral occlusion is applied to rats loaded with mannitol in order to magnify the transmembrane gradient of PAH at the site of secretion. The concentrations in lumen, cell, and interstitium are determined by simultaneous analysis of intraluminal fluid (micropuncture technique), of cortex homogenate, and of plasma.

Data obtained agree essentially with the hypothesis that PAH is actively transported in the peritubular membrane of the renal proximal tubule cells.

Methods. Following 24 hr of water and food deprivation, Sprague-Dawley rats (200–250 g) were anesthetized with Inactin (100 mg/kg). The left kidney was exposed for micropuncture, its ureter was catheterized, and it was mounted in a Lucite cup by conventional methods. The ^{3}H-PAH (200 μCi) and ^{14}C-inulin (50 μCi) were injected intramuscularly.

Osmotic diuresis was induced by intravenous infusion of a solution containing 1.0 *M* mannitol, 0.03 *M* NaCl, and 0.01 *M* KCl at a rate of 5–6 ml/hr. The radioactive substances were administered as soon as a diuretic response was established in the exposed kidney. Almost steady-state plasma levels of these substances were achieved 30–40 min after injection.

Following collection of 2–4 samples from proximal and/or distal tubules by the micro-

[1] Supported by U.S. Public Health Service Grant AM 12501-01A1.

[2] Recipient of Research Career Program Award 5-K3-AM-35,248 from the National Institutes of Health.

[3] Recipient of Research Fellowship Award 1-F2-Am-38,385 from the National Institutes of Health.

puncture technique, the ureter was ligated (or the catheter was clamped). Preceding ligation of the ureter about 0.1 ml of urine was injected retrogardely in order to distend the renal pelvis and accomplish instantaneous mechanical obstruction. Distal tubules were identified by intravenous injection of Lissamine green (11). One or two intravenous injections of 0.2 to 0.3 ml of 1.4% solution of Lissamine green were sufficient for the free-flow period. The distal tubules remained green (but not the proximal tubules) during the entire period of obstruction (30–40 min) when 0.3 to 0.4 ml were injected 0.4 to 0.5 min preceding ureteral obstruction. Two to five micropuncture samples were obtained during the period of obstruction from proximal and/or distal tubules.

The experiment was terminated at the end of the stop-flow period and both kidneys were removed without release of the obstruction. The right kidney was used as the free-flow control. The cortex was quickly excised and frozen in liquid nitrogen for analysis of its homogenate.

Two to three samples of urine were collected preceding ureteral obstruction. Blood samples were obtained between each pair of micropuncture samples. The ^{14}C and ^{3}H concentrations in micropuncture samples, plasma, urine, and cortex homogenates were determined by differential counting in a liquid scintillation counter. The lowest counts were obtained in some micropuncture samples and they were at least 2–3 times higher than the background. The counting time was at least 30 min/sample.

Statistical analysis was carried out by using Student's *t* test. The method of paired comparisons was used when possible.

Special considerations on the micropuncture technique used. The purpose of the micropuncture technique used was to obtain the average concentrations of ^{3}H-PAH and ^{14}C-inulin in the lumen of the proximal and distal tubules, in order to compare them to the average concentration in the cortex homogenate. Therefore, although it was important to know whether the punctured tubule was proximal or distal, the exact site of puncture was not necessary. In addition, to localize the exact site of puncture by latex injection (12) would have been an insurmountable task because of the large number of tubules punctured in each experiment (up to 60 or 70). Nevertheless, we had a high degree of certainty of whether the tubules punctured were proximal or distal by using Lissamine green. Thus, a series of preliminary experiments were performed as described above (without isotopes) to test our ability to identify the tubules with Lissamine green alone. Following injection of the dye, selected tubules were injected with latex (12), the kidney was digested with hydrochloric acid and the latex-injected tubules were dissected for identification. The correlation between identification with the dye and with latex was close to 100%, for both the free-flow and stop-flow periods of the experiment. These preliminary experiments were used also to test the amount of Lissamine green needed during the free-flow and stop-flow periods; and the time of injection of the dye with reference to obstruction, in order to obtain optimal identification during the period of stop flow. The amounts and time of injection used in the actual experiments (see above) were found to be the most satisfactory.

The fluid from proximal or distal cortical tubules had to be obtained with minimal contamination from other structures, in order for the sample to represent the fluid in that region at the time of collection. During the free-flow period this was not a major problem as long as the sample was collected at a rate below 20 nl/min, the normal nephron flow.

During the stop-flow period, in order to get enough fluid for analysis, several tubules had to be punctured. The pooled sample size varied from 20 to 130 nl although most samples were between 50 and 70 for proximal tubules and 70–90 nl for distal tubules. Contamination by new filtrate, or by retrograde collection from distant regions of the nephron, was held to a minimum during ureteral obstruction because of the following: when a tubule is punctured and collections are made, while the ureter is obstructed, two phases of collection rate are seen; a very fast early phase, followed by a slower phase. In proximal tubules, these two phases probably represent the rapid filling of the collection pipette by fluid trapped all along the prox-

TABLE I. Effect of Ureteral Obstruction on Proximal Tubule ^{14}C-Inulin and ^{3}H-PAH in Mannitol Diuresis.[a]

Rat	^{3}H-PAH		^{14}C-Inulin	
	FF	StF	FF	StF
A	1.6 (1)	33.9 (3)	1.3 (1)	1.6 (3)
B	—	20.4 (4)	—	2.2 (4)
C	2.3 (3)	19.6 (4)	1.1 (3)	2.3 (4)
D	4.6 (3)	18.3 (4)	1.6 (3)	3.0 (4)
E	6.4 (2)	21.5 (5)	3.0 (2)	3.9 (5)
F	1.9 (2)	18.7 (4)	1.0 (2)	3.7 (4)
G	2.4 (3)	22.0 (3)	1.0 (3)	2.8 (3)
H	6.8 (4)	33.2 (4)	2.7 (4)	2.6 (4)
I	5.7 (4)	28.9 (5)	2.6 (4)	3.7 (5)
J	4.7 (3)	29.3 (5)	3.0 (3)	4.6 (5)
K	3.7 (2)	15.6 (5)	2.4 (2)	4.0 (5)
L	—	—	—	5.9 (1)
M	2.2 (2)	35.7 (1)	3.4 (2)	4.5 (1)
Rat mean	3.8 (11) ±0.6	28.1 (12) ±3.8	2.1 (11) ±0.3	3.4 (13) ±0.3
Sample mean	4.2 (29) ±0.6	24.8 (48) ±1.8	2.2 (29) ±0.2	3.3 (48) ±0.2

[a] FF = free-flow period; StF = stop-flow period. Values are the fluid to plasma concentration ratios. The values in the rows headed by a letter are means and the number of samples obtained during the experimental period. Rat mean = the mean of means from each rat. Sample mean = the mean of all samples collected during the experimental period.

imal tubule, including the pars recta; the slow phase probably results from delivery of fluid by renewed filtration. In distal tubules, the fast early phase probably represents the rapid filling of the collection pipette by fluid trapped in the four or five distal tubules that are tributaries to the cortical collecting duct to which the punctured tubule contributes; the slow phase probably results from renewed filtration. That several distal tubules contribute to the puncture of one is suggested by the fact that the first phase of stop-flow collection was faster and larger in distal than in proximal tubules despite the fact that the volume of individual nephrons is larger in proximal than in distal tubules. *Only the early gush of fluid was collected from each tubule.*

Results. Experiments were performed on 21 rats loaded with mannitol. The mean (±SEM) urine flow during the free-flow period was 3.65 (±0.30) ml/hr for the left kidney. The mean (±SEM) clearances of ^{14}C-inulin and ^{3}H-PAH were 33.3 (±3.0) and 98.0 (±7.3) ml/hr, respectively, for the same kidney. The approximate mean chemical concentration of PAH was 10^{-7} to 10^{-6} *M* in plasma.

Proximal tubules. Table I presents compiled data obtained by micropuncture of proximal convoluted tubules in 13 experiments. The values in the two columns are the mean values of the fluid to plasma concentration ratios for the samples collected from each rat, before the ureter was obstructed and during the period of obstruction.

The "rat mean" was obtained from the mean value of each rat. The "sample mean" is the mean of all the samples obtained during the given experimental period.

The tubular fluid to plasma concentration ratios (TF/P) of ^{3}H-PAH and ^{14}C-inulin were consistently higher in the samples obtained during the period of ureteral obstruction than in the preobstructive samples ($p<0.01$).

Figure 1 shows the data obtained from the same rats as in Table I. The TF/P ratios are plotted versus time. The zero free-flow time represents the time of administration of the

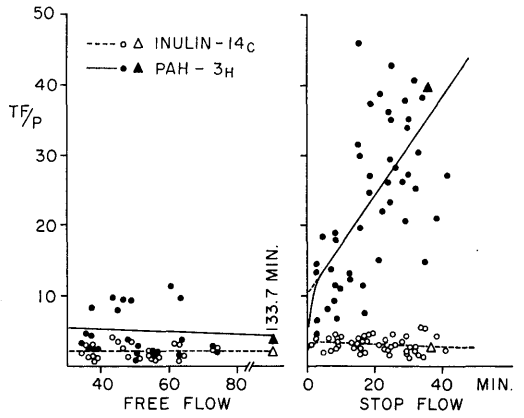

FIG. 1. Proximal tubule fluid (or cortex homogenate) to plasma concentration ratios from 13 rats in mannitol diuresis: (○,△) ^{14}C-inulin; (●,▲) ^{3}H-PAH. (○,●) individual micropuncture samples; (△,▲) the mean cortex homogenate to plasma ratio plotted versus the mean time of removal of the stop-flow kidney. The time on the abscissa is: (a) free flow = time of injection of isotopes; and (b) stop flow = time of ureteral obstruction.

radioactive isotopes; the stop-flow time is presented in reference to time of obstruction of the ureter.

Continuous lines are the calculated regression lines for ^{3}H-PAH; broken lines are the calculated regression lines for ^{14}C-inulin. The slopes of the two ^{14}C-inulin regression lines and of the ^{3}H-PAH regression line during free flow are not different from zero. The slope of the stop-flow regression line for ^{3}H-PAH is significantly different from zero ($p<0.05$), and equals 0.7 ml of plasma PAH/ml of tubular fluid/min. Although there is no apparent increase in the ratios from 20 to 40 min, this is apparently due to the fact that data varies from one rat to another. Thus, the last sample collected was higher than previous samples in 7 out of 11 experiments with more than one stop-flow collection.

In any event, while the ^{14}C-inulin concentration slightly increased only in the beginning of the ureteral obstruction period, the ^{3}H-PAH concentration kept increasing from a TF/P value of about 4 during the period of free flow to values as high as 40 to 45. It should be pointed out that these data rule out the possibility of very large contamination of proximal samples with fresh filtrate. A marked contamination would have resulted in a nonsignificant increase in the concentration of ^{3}H-PAH during stop flow.

Distal tubules. Table II presents compiled data obtained by micropuncture of distal convoluted tubules in seven rats.

The TF/P ratios for ^{3}H-PAH increased during stop flow in all but one rat (N) and the increase was significant ($p<0.01$) when the sample mean were used in the analysis. The TF/P ratios for ^{14}C-inulin decreased during stop flow in all but one rat (P) and the decrease was significant ($p<0.05$). The stop-flow decrease in concentration of ^{14}C-inulin is puzzling since the obvious explanations, a leak of inulin out or of water into the tubule, are very unlikely to occur in the distal tubule.

Figure 2 shows the data obtained from the same rats as in Table II and the plot is the same as in Fig. 1. As in the proximal tubules (Fig. 1), the slopes of the two ^{14}C-inulin regression lines and the slope of the ^{3}H-PAH regression line during free flow are not significantly different from zero. During stop flow the regression line for ^{3}H-PAH is significantly different from zero ($p<0.05$) and equals 2.4 ml of plasma PAH/ml of tubular fluid/min.

Since, as shown by Cortney *et al.* (1), there is no secretion of PAH in the distal tubule, the increase in ^{3}H-PAH concentration during ureteral obstruction in distal tubule samples is apparently due to a slow movement of fluid from proximal to distal tubule. The increase in ^{3}H-PAH concentration could also be due to contamination of the collection with proximal tubular fluid, but this is unlikely because : (a) while fluid from distal

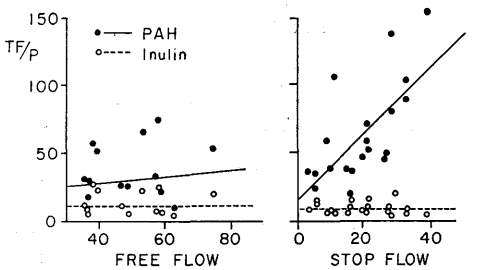

FIG. 2. Distal tubule to plasma concentration ratios from seven rats in mannitol diuresis. Format is the same as that of Figure 1.

TABLE II. Effect of Ureteral Obstruction on Distal Tubule ^{14}C-Inulin and ^{3}H-PAH in Mannitol Diuresis.[a]

Rat	^{3}H-PAH		^{14}C-Inulin	
	FF	StF	FF	StF
L	26.8 (2)	42.5 (5)	11.4 (2)	8.9 (5)
M	13.4 (2)	34.4 (4)	9.0 (2)	8.2 (4)
N	58.6 (3)	41.4 (3)	22.2 (3)	13.2 (3)
O	62.2 (2)	76.3 (2)	24.5 (2)	18.3 (2)
P	13.6 (2)	45.2 (4)	5.8 (2)	6.1 (4)
Q	24.2 (2)	132.4 (3)	5.8 (2)	4.2 (3)
R	32.4 (2)	73.5 (3)	7.7 (2)	5.1 (3)
Rat mean	33.0 (7) ±7.5	63.7 (7) ±13.0	12.4 (7) ±2.9	9.2 (7) ±1.9
Sample mean	34.7 (15) ±5.2	59.4 (24) ±7.3	13.0 (15) ±2.0	8.6 (24) ±0.8

[a] Abbreviations and values same as in Table I.

tubules was collected quickly without any suction, proximal tubules yielded fluid at a lower rate and a slight suction was sometimes necessary to initiate the collection; and (b) only the early gush was obtained from 4 to 10 tubules punctured in each collection.

In any event the fact that the increase of ^{3}H-PAH concentration in the distal tubule during stop flow was higher than in the proximal tubule strongly suggests a high level of ^{3}H-PAH secretion somewhere between the proximal and distal convolutions. Our data support the observations made by Tune *et al.* (13) that PAH secretion was 3–4 times higher in the straight portion than in the convoluted portion of the proximal tubule when the peritubular concentration was 2.4×10^{-5} *M*.

Cortex homogenate. Table III presents summarized data on cortex homogenate to plasma concentration ratios (CH/P) from six rats that were nephrectomized at the termination of the experiments, without release of the ureteral obstruction. While ^{14}C-inulin concentration increased slightly but not significantly with ureteral obstruction ($0.1 < p < 0.2$), a marked increase in the ^{3}H-PAH concentration was observed during that period, from a CH/P ratio of 3.34 to one with a value of 39.8 ($p < 0.01$).

TABLE III. Effect of Ureteral Obstruction on Renal Cortex Homogenate.[a]

	(ml plasma/ml tissue water)			
	^{3}H-PAH		^{14}C-Inulin	
	FF	StF	FF	StF
Mean	3.34 ±0.38	39.8 ±5.5	1.74 ±0.21	2.21 ±0.45

[a] FF = free-flow kidney; StF = stop-flow kidney. Values are the mean ± SEM of the tissue water to plasma concentration ratios in six rats.

Stop-flow accumulation of ^{3}H-PAH. By subtraction of the TF/P (or CH/P) ratios obtained during the free-flow period from the TF/P (or CH/P) ratio during the stop-flow period a change in concentration is obtained in milliliters of plasma per milliliter of tubular fluid (or per milliliter of tissue water). A positive value will denote stop-flow accumulation of the substance.

In order to compare stop-flow accumulation in cortex homogenate with that in the tubule, values should be obtained at equal duration of ureteral obstruction. Therefore, the mean time of removal of the stop-flow kidney can be used to obtain the stop-flow TF/P ratios for proximal and distal tubules from their regression lines.

Table IV presents data on accumulation of ^{3}H-PAH in proximal tubules, distal tubules, and cortex homogenates, at the time of 36.0 min stop flow. This was the mean time of removal of the stop-flow kidney (SEM = ±1.8 min). Stop-flow accumulation of PAH

TABLE IV. Stop-Flow Accumulation of ^{3}H-PAH in Mannitol Diuresis.[a]

$(TF/P)_{StF}-(TF/P)_{FF}$.

Proximal tubule (lumen)	Distal tubule (lumen)	Cortex homogenate
31.6	66.5	36.4

[a] Units are in milliliters plasma ^{3}H-PAH per milliliter lumen (or tissue) water, per 36 min of ureteral obstruction (see text).

was highest in distal tubules (66.5 ml of plasma/ml of tubular fluid) followed by cortex homogenates (36.4) and proximal tubules (31.6).

Discussion. The fact that stop-flow accumulation of ^{3}H-PAH was equal or higher in cortical tissue water (36.4 ml plasma/ml total tissue water) than in the lumen of the proximal tubule (31.6 ml plasma/ml luminal water), strongly supports the hypothesis that PAH secretion in the proximal tubule takes place by a "push" system, that is, with the active pump located in the peritubular membrane. The proximal cell, being a major fraction of the cortical region, has to be a major contributor to the high ^{3}H-PAH accumulation in the whole homogenate. Could the distal tubule be a major contributor to the whole homogenate since the stop-flow accumulation of ^{3}H-PAH was 66.5 ml plasma/ml luminal water in this compartment? In order to answer this question, we should consider the fact that stop-flow accumulation of ^{3}H-PAH should not take place in the interstitium since the blood flow continues to be effective during the period of ureteral obstruction (14). The interstitium (including the vascular compartment) contributes at least 20% to the total tissue water (15–17). Therefore, whatever contribution of the "accumulating" compartments to the total cortical tissue will be diluted by the interstitial water. The distal luminal water contributes quite less than 10% to the total tissue water.[4] Although accumulation in distal lumen was about twice as high as in the lumen of the proximal tubule or in total homogenate, this would still be insufficient to compensate for the diluting effect of the interstitial space. Therefore, another compartment has to contribute to the total tissue accumulation of PAH, and the only compartment left is the cells of the proximal tubule. As a matter of fact, stop-flow accumulation in the cells of the proximal tubule has to be somewhat higher than in the lumen in order to compensate in part for the diluting effect of the interstitium.

Despite the precaution taken to avoid contamination of micropuncture samples by fresh filtrate or by retrograde collection (see methods), a minimal contamination might have been unavoidable. Nevertheless, this contamination must have been of an insignificant nature: (i) While the ^{3}H-PAH concentration kept increasing significantly during the period of ureteral obstruction the concentration of ^{14}C-inulin remained unchanged. An increase in ^{3}H-PAH concentration because of retrograde collection should have been accompanied by a parallel increase in the ^{14}C-inulin concentration. (ii) With a significant contamination with new filtrate it would be very unlikely to obtain a significant stop-flow accumulation of ^{3}H-PAH, which kept increasing as the duration of stop-flow increased.

Essentially (a) the concentration gradient of ^{3}H-PAH between interstitium and cell was accenuated by ureteral obstruction, with the concentration in the cell being higher than in the interstitium; and (b) the concentration of ^{3}H-PAH in the proximal cell remained equal or higher than in the proximal lumen during the stop-flow period. Therefore, PAH seems to be actively transported across the peritubular membrane of the proximal

[4] One can arrive at the conclusion that the distal tubule contributes less than 20% and the distal lumen to much less than 10% to the total cortical water since: (i) the interstitial water (including intravascular water) contributes at least 20% to total cortical water (15-17); (ii) the length of proximal tubules is about four times that of the distal tubules (18); (iii) the diameter of the proximal and distal tubules are very similar in mannitol loaded rats (19); and (iv) the distal cell compartment (outer concentric cylinder) should be larger than the distal luminal compartment (inner cylinder) since the length of the radius of the latter is about the same as the thickness of the cell compartment.

cell and may move passively across the luminal membrane. Although other authors (5–8) have arrived at the same conclusion, our present data are the first to reflect *in situ* the concentration profile through the path of PAH secretion, that is, from interstitium to cell, into the lumen. This conclusion is strongly supported by the fact that the cell is negatively charged to both interstitium and lumen (20), which further would complicate the entrance of PAH (negative ion) into the cell but would facilitate its passive movement into the lumen. High cellular concentrations of ^{3}H-PAH have been observed by Tanner and Kinter (21) using the autoradiographic technique in the necturus kidney. In this species PAH and Diodrast are apparently both actively secreted and reabsorbed. The authors suggested that a "push" system exists for both processes, that is, the secretory pump is in the peritubular membrane and the reabsorptive pump in the luminal membrane.

Why should accumulation in cell be so much greater than expected from electrochemical equilibrium? If a simple passive system for PAH exists between cell and lumen in the rat, one would expect the following chain of events to occur during ureteral obstruction: stagnation of fluid in the proximal tubule would induce an increase in the concentration of PAH in the lumen until electrochemical equilibrium exists between cell and lumen. Only at this point the concentration in cell should begin to rise, with further increase in the lumen to maintain the equilibrium. Consequently, one would expect a higher stop-flow accumulation of PAH in the lumen than in the cell if a simple passive diffusion exists in the luminal membrane. Perhaps, the movement of PAH from cell to lumen would take place by "facilitated diffusion" (22). The PAH carrier could be shared by some substance which is normally filtered and reabsorbed in a fashion somewhat similar to the phenomenon of "counterflow" (23). During stop flow, impaired filtration would diminish the supply of reabsorbable substance to the carrier site, impairing the exit of PAH. If the resulting net transport of PAH across the luminal membrane became lower than the net entry by active transport through the peritubular membrane, PAH would accumulate in the cell. Accumulation in the lumen would still be the result of stagnation with a lower movement of PAH along the nephron than its net movement across the luminal membrane.

The fact that stop-flow concentration of ^{3}H-PAH increased to higher levels in samples obtained from distal tubules than in samples obtained from proximal tubules suggests strongly that there is a region between the proximal and distal convolutions where ^{3}H-PAH is secreted at a relatively high rate. These data support the observation made by Tune *et al.* (13) that PAH secretion takes place in the straight portion of the isolated rabbit proximal tubule.

Summary. Stop-flow accumulation of ^{3}H-PAH took place in cells and in lumina of proximal tubules. This strongly suggests that an active PAH is secreted by a "push" system, that is, the pump is located in the peritubular membrane of the cell. The rate of stop-flow accumulation of ^{3}H-PAH in the distal tubules was over three times higher than in proximal tubules, while the concentration of ^{14}C-inulin was decreasing in the distal tubules. Apparently there is a very active secretory pump for PAH between the proximal and distal convolutions, probably in the straight portion of the proximal tubule.

The authors thank Dr. Stanley Ignatow for his collaboration during a summer predoctoral research fellowship; and Miss Doris G. Fravert for her technical assistance.

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Received Nov. 23, 1970. P.S.E.B.M., 1971, Vol. 137.