Local and Systemic Effects of Phenylalanine on Intestinal Transport of Tyrosine and Tryptophan[1] (36525)

RAUL A. WAPNIR, ROBERT L. HAWKINS, AND FIMA LIFSHITZ
(Introduced by H. C. Harrison)

Department of Pediatrics, University of Maryland School of Medicine, Baltimore, Maryland 21201 and Rosewood State Hospital, Owings Mills, Maryland 21117

L-Phenylalanine (Phe) has been shown to inhibit the intestinal transport of L-histidine (1), L-tyrosine (2) (Tyr), and L-tryptophan (3) (Trp). Phe also reduces the uptake of other amino acids by brain (4–6), and therefore, it may play a role in the brain damage of phenylketonuric (PKU) patients (4, 5).

PKU patients with elevated plasma Phe levels have lower blood concentrations of other circulating amino acids (7). The intestinal transport of labeled arginine and leucine (8), as well as Trp (9), given as an oral load, is diminished. However, the renal tubular reabsorption of Phe and of 15 other amino acids is not altered by the presence of elevated circulating Phe levels (10).

The local and the systemic effects of Phe on the intestinal transport of Tyr and Trp by everted intestinal loops *in vitro* and by perfusion of intestinal segments *in vivo* were studied in rats. Phe inhibited the absorption of these amino acids when it was present in the buffer in a 10:1 ratio. However, hyperphenylalaninemia induced by feeding excessive amounts of Phe or by chronic ingestion of a Phe hydroxylase inhibitor did not alter the intestinal transport of Tyr and Trp.

Materials and Methods. Experiments were conducted with female Wistar rats with a mean initial weight of 103.3 ± 10.3 g (± 95% CI). For each experiment, the rats were randomly divided into groups and were studied following a period of one week on their respective diets. The control rats (C) were fed powdered Purina Lab Chow containing 1.2% Phe. A high phenylalanine group (HiPhe) received the same feed with an additional 7% (w/w) of this amino acid.[2] A third group was given Purina Lab Chow to which 0.1% DL-p-chlorophenylalanine (p CPA)[2] had been added. This supplement has been shown to suppress nearly 90% of the liver Phe hydroxylase activity (11). A fourth set of animals received exclusively a powdered product with no more than 0.1%. Phe, Lofenalac[3] (LoPhe). All rats were fed water *ad libitum* and their respective diet until the experiment was terminated. After 1 week the C group had attained an average weight of 112.7 ± 11.1 g; the HiPhe, 92.9 ± 7.8 g; the pCPA, 108.9 ± 13.4 g and the LoPhe, 85.5 ± 9.3 g ($p < .05$).

Two types of experiments were performed. The transport of Tyr and Trp was measured by everted intestinal loop preparations *in vitro,* and by perfusing an intestinal loop *in vivo* as described below. Measurements of the *in vitro* concentrative transport (12) of Tyr and Trp were made in everted intestinal loops taken from rats anesthetized with sodium barbital (50 mg/kg body wt), administered intraperitoneally. The small intestine was removed and after washing it with three 10-ml portions of cold saline, seven everted loops were made from each 8 cm segment, beginning at the pylorus. The first segment was discarded since only minimal amino acid transport occurred.

The intestinal loops were placed in beakers containing 5 ml of Krebs–Henseleit bicarbonate buffer, 20 m*M* in glucose with addi-

[1] Read in part at the F.A.S.E.B. 55th Annu. Meet. Apr. 13, 1971, Chicago, IL.

[2] Sigma Chemical Company, St. Louis, MO. 63118.

[3] Mead Johnson Laboratories, Evansville, IN 47708.

tion of the appropriate amino acid, L-Tyr and L-Trp in varying concentrations. Phe was also added in equimolar concentration in randomized alternated intestinal loops. In some experiments, Phe was added in a 10:1 ratio to the other amino acids. In all instances the buffers were kept isotonic by the addition of appropriate amounts of sodium chloride. Six tenths milliliter of the same buffer present in the outside solution was introduced with a tuberculin syringe into the serosal compartment. The intestinal loops were incubated for 1 hr at 37° in a Dubnoff apparatus under an atmosphere of O_2:CO_2 (95:5). At the end of this period, the intestinal loops were blotted and the contents of both serosal and mucosal compartments were recovered quantitatively. Glucose and amino acid concentrations were determined. The index of transport efficiency was the ratio of the concentration of solute in the serosal fluid to that of the mucosal fluid at the end of the incubation (C_s/C_m).

For the perfusion studies *in vivo* the rats were anesthetized with 1.5 g/kg body weight of urethane administered subcutaneously. The abdominal cavity was opened by a midline incision and the small intestine was cannulated below the ligament of Treitz. A 20-cm long segment was utilized and the intestinal contents were washed with two 10-ml portions of saline. The proximal junction was attached to a Harvard peristaltic pump and perfused at a rate of 0.17 to 0.20 ml/min. The solutions used were Krebs–Henseleit bicarbonate buffers, 20 m*M* in glucose, with the addition of the appropriate amino acid in the same concentrations as in the *in vitro* studies. In some experiments, glucose content was increased to 139 m*M*. In all instances, polyethylene glycol, mol wt 3000–3700 (PEG, Carbowax 4000)[4] was added to the buffers at a concentration of 400 to 600 mg/100 ml and were made isotonic by the addition of sodium chloride. These were kept in a 37° water bath during the perfusion and bubbled with O_2:CO_2 (95:5) gas. Fractions were collected every 15 min for 1 hr after a 45-min equilibration period. A 15 min wash period was allowed before collecting samples for a second hour. The order of buffers perfused (with and without Phe) was randomized. The passage time for the solutions pumped was determined to be 9 to 10 min using phenol red as a nonabsorbable indicator. Glucose and amino acid concentrations were determined in each 15 min sample and the amount of each absorbed was calculated (13). The variations between the samples were minimal ($p > .05$) and, therefore, the results were grouped into a single mean per hour for each solute. The rate of absorption was expressed as millimicromoles of the solute absorbed per minute, per centimeter of intestine.

Hyperphenylalaninemia was induced in both the HiPhe- and pCPA-treated animals. For the *in vitro* experiments, blood was obtained from the abdominal aorta at the time the intestine was removed. The HiPhe animals had a mean blood Phe level of 30.4 ± 15.1 mg/100 ml (± 95% CI) and the pCPA rats had a total Phe mean of 19.1 ± 8.8 mg/100 ml. For the *in vivo* studies, blood was obtained from the tail at the beginning of the experiment, and at the end from the abdominal aorta. Initially, the HiPhe rats had high circulating levels, mean 63.2 ± 12.3 mg/100 ml, which rapidly fell. Therefore, only those animals maintaining blood Phe levels above 10 mg/100 ml at the end of the perfusion period were considered in this group. The rats which did not meet this criterion were eliminated. The pCPA group had hyperphenylalaninemia undergoing no changes over the span of the experiment. Total Phe had a mean of 22.4 ± 4.0 mg/100 ml, the pCPA fraction was 46% of the total Phe. The C and LoPhe groups always had blood Phe levels below 3 mg/100 ml.

The following analytical methods were used: Total Phe in blood by the method of Wong, O'Flynn and Inouye (14). The pCPA fraction of total Phe in blood was determined by high voltage electrophoresis at pH 1.9, 2000 V for 1 hr, the bands were stained with ninhydrin, eluted with methanol and quantitated by spectrophotometry (15). In the perfusates, Trp was measured by a microspectrofluorometric assay (16) and Tyr also by spectrofluorometry (14). Glucose was as-

[4] Allied Chemical Corp., Clifton, NJ 07013.

TABLE I. Local Effect of Phenylalanine on the Intestinal Transport of 1 m*M* Tyr and 5 m*M* Trp *in Vitro*.[a]

Additions	C_s/C_m	
	1 m*M* Tyr	5m*M* Trp
No phenylalanine	5.00 ± 1.20 (24)	1.22 ± 0.12 (10)
Phenylalanine 1:1	4.21 ± 0.80 (24)	1.09 ± 0.10 (9)
10:1	2.60 ± 1.00[b] (12)	0.94 ± 0.11[b] (12)

[a] Everted jejunal loops from C rats were incubated in Krebs–Henseleit buffer with 20 m*M* glucose. The concentration ratios (C_s/C_m) achieved by intestinal loops incubated for 1 hr in the absence or presence of Phe in a 1:1 or 10:1 molar ratio are shown. All buffers remained isotonic by the addition of sodium chloride. The transport of glucose was similar in all loops. Data are means ± 95% CI. (n) = number of loops.

[b] The differences in amino acid transport were significant for the high Phe buffer ($p < .05$).

sayed with glucose oxidase (17) and PEG by the method of Malaver and Powell (18).

The amino acid content of the intestinal mucosa was determined on 1:5 homogenates of mucosa scrapings obtaned from intestinal segments before or after the perfusion. Protein was assayed by the Lowry *et al.* (19) procedure. The experiments were performed on a 2 × 2 factorial design. The statistical analysis of the means was made by a one or a two variable classification (20).

Results. The local effect of Phe on Tyr and Trp transport *in vitro* is shown in Table I. The presence of Phe in equimolar concentrations in the buffer did not inhibit the transport of these amino acids by everted intestinal loops. However, when the concentration of Phe in the buffer was increased to a 10:1 ratio, there was a 48.2% inhibition for Tyr and 23.3% for Trp. These findings are in agreement with previously reported studies (2, 3).

Hyperphenylalaninemia produced no changes on Tyr and Trp transport *in vitro*. There were no differences in the transport of these amino acids between the intestinal loop preparations from rats fed C, HiPhe, pCPA, or LoPhe diets. The concentration ratios of serosal to mucosal phases (C_s/C_m) achieved by everted intestinal loops of rats with high circulating Phe levels were similar to those of animals with normal blood Phe, both in the preparation containing equimolar concentrations of Phe in the buffer as well as those without this amino acid. The concentration ratios of each of these amino acids attained by all groups of animals were within the range reported by others (2, 3), with an overall C_s/C_m mean of 1.25 ± 0.04 for Trp and 4.99 ± 0.54 for Tyr.

The local effect of Phe on Tyr and Trp transport *in vivo* is shown in Figs. 1 and 2. The transport of these amino acids by intestinal segments was the same when perfused with buffer solutions containing equimolar concentrations of Phe as that attained without it. However, the transport of these amino acids was significantly decreased when the perfusate contained Phe in a 10:1 ratio (Fig. 2). There was an inhibition of 36% for Tyr and 37% for Trp. Since the rate of glucose transport was not altered, the inhibition of amino acid absorption was specific.

The possibility that lower amino acid transport was the result of decreased sodium concentration in the buffer was studied by substituting mannitol for Phe at 50 m*M* concentrations. At constant sodium concentration, Phe altered amino acid intestinal absorption as shown in Fig. 2. Mannitol in the buffer had no effect on Tyr and Trp absorption.

The systemic effect of hyperphenylalaninemia on 1 m*M* Tyr and 5 m*M* Trp absorption *in vivo* is shown in Fig. 1. These concentrations have been utilized for studies of the amino acids in everted intestinal loops *in vitro* (2, 3) and *in vivo* (13, 21). There were no differences in the amount of Tyr and of Trp absorbed by rats fed C, HiPhe or pCPA diets. The rates of transport of these amino acids achieved by rats with hyperphenylalaninemia were the same as those with normal blood Phe. Rats fed a LoPhe diet had a decreased absorption of Tyr. An increase in the concentration of glucose from 20 to 139 m*M* in the perfused buffer produced no changes in Tyr or Trp absorption.

Since the concentration of Tyr and Trp perfused was higher than the circulating

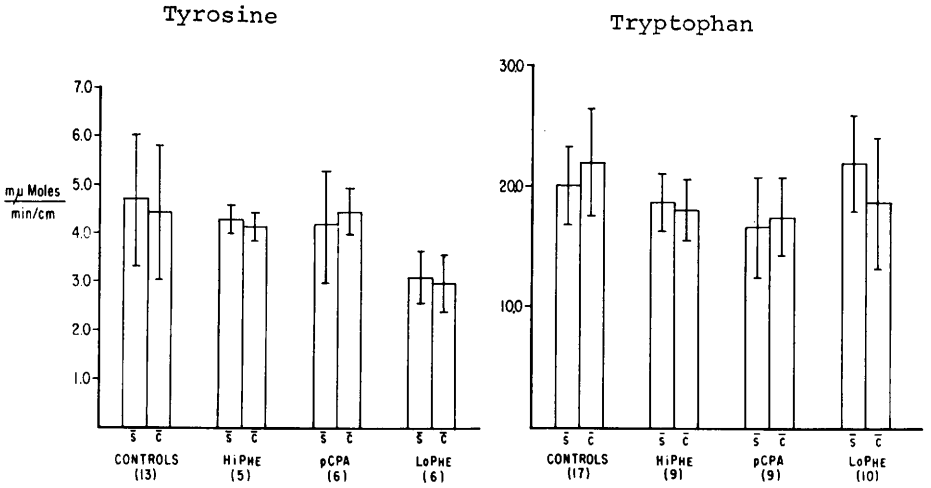

FIG. 1. Influence of Phe on the absorption of 1 m*M* Tyr and 5 m*M* Trp *in vivo*. Data show amounts of those amino acids absorbed by rats fed C, HiPhe, pCPA and LoPhe perfused with buffers with ($\bar{c}$) or without ($\bar{s}$) an equimolar concentration of Phe. The order of perfusion of the two buffers was randomized. The concentration of glucose was 20 m*M* and the solutions were isotonic. The height of the columns represents the amount of Tyr and Trp absorbed (mμmoles/min/cm). The differences in the rate of transport of Tyr were significant only for the LoPhe group ($p < .01$). This effect may be related to the poor nutritional status of the rats. There were no differences in the rate of Trp absorption regardless of the presence or absence of Phe in the buffer. C = diet containing 1.2% Phe. HiPhe = diet containing 8.2% Phe. pCPA = C diet plus 0.1% pCPA. LoPhe = diet containing 0.1% Phe. (n) = number of rats.

blood levels of these amino acids, additional experiments were conducted perfusing 0.1 m*M* Tyr and Trp in isoosmolar, Krebs–Henseleit buffer with 20 m*M* glucose. Hyperphenylalaninemia did not result in inhibition of intestinal transport of either amino acid. The mild hypertyrosinemia of the HiPhe group (mean Tyr in HiPhe rats: 6.05 mg/100 ml; in C rats: 1.65 mg/100 ml did not alter the intestinal transport of Tyr. The HiPhe rats had unchanged blood Trp levels.

The amino acid content of the mucosa (Table II) showed no significant differences among the three groups of rats. In particular, neither the HiPhe or pCPA diets produced an increase on the free Phe concentration in the tissue cells in immediate contact with the perfusates.

The capacity of the intestinal segment for glucose transport *in vivo*, at 20 m*M* concentration, was far below the maximum absorption. An increase in the concentration of the carbohydrate to 139 m*M* produced a proportional increase in the absorption of glucose (Table III). There were no differences in the rate of carbohydrate absorption regardless of the amino acid perfused simultaneously and the presence or absence of Phe in the buffer solution. The rate of glucose transport achieved by rats with hyperphenylalaninemia was the same as that of animals with normal blood Phe. The glucose transport rates attained in these experiments were similar to those reported for similar glucose concentrations and infusion rates (22).

Discussion. Our experiments indicated that hyperphenylalaninemia, even of a severe degree, did not interfere with the absorption of Tyr and Trp from the intestine. These experimental findings are in agreement with the report of Brodehl, Gellissen and Kaas (10) who showed that hyperphenylalaninemia in untreated PKU patients did not interfere with the renal tubular reabsorption of Phe and 15 other amino acids.

However, Phe exerts a local inhibitory effect on the intestinal transport of L-histidine (1), L-Tyr (2) and L-Trp (3) when the

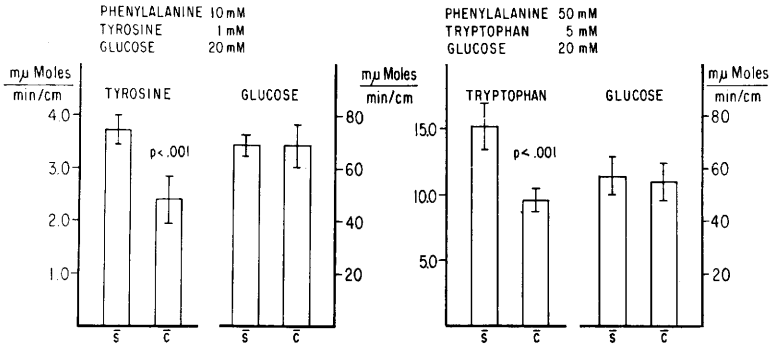

FIG. 2. *In vivo* absorption of amino acids and glucose in the presence or absence of 10:1 concentrations of Phe. Tyr was perfused at 1 m*M* concentration, with or without 10 m*M* Phe. Trp was given at a 5 m*M* level, with or without 50 m*M* Phe. An equimolar concentration of mannitol substituted Phe in the buffers without this amino acid. All perfusing fluids containing 20 m*M* glucose. The results are expressed as mμmoles/min/cm. The differences between buffers with or without Phe were highly significant ($p < .001$) for amino acid transport. Data are means ± 95% CI. Four rats on C diet were used for each amino acid.

ratios of Phe in the buffer to the other amino acids are above one. Lin and Wilson (2), using everted intestinal sacs from hamsters, showed a 15% inhibition in the transport of Tyr when the ratio of Phe:Tyr concentrations was 5:3. For 15 m*M* and a 20 m*M* Phe concentration in the presence of a 3 m*M* Tyr the inhibition increased above 80%. Spencer and Samiy (23) found no inhibition of Trp transport in the presence of equimolar Phe at 5 m*M* concentrations *in vitro*. In our experiments there was a significant reduction of Tyr and Trp transport by everted intestinal loop preparations from rats *in vitro* and by perfusion *in vivo*, when the Phe:Tyr and Phe:Trp ratios were 10:1. On the other hand, the presence of equimolar concentrations of Phe did not alter the rate of transport of those amino acids, both *in vitro* and *in vivo*.

The elevated blood Phe concentration induced by feedings of excessive amounts of Phe in the diet did not attain the molarity that was shown to inhibit the intestinal transport of those amino acids when present in the buffer. Blood Phe concentrations between 2 to 4 m*M* appear to be too low to create at the intestinal mucosal level a gradient which would interfere with the normal uptake of the other amino acids. However, these circulating blood Phe concentrations are generally not exceeded by untreated PKU patients or even in cord blood obtained from experimental hyperphenylalaninemic mammals (24). This agrees with the lack of inhibitory effect when Phe was present in the buffer in equimolar concentrations.

Rats fed pCPA also showed no impairment in their intestinal absorption rates for Tyr and Trp. This result may exclude the possibility that hyperphenylalaninemia resulting from Phe hydroxylase inhibition would affect the transport of other aromatic amino acids. Moreover, since the inhibition of liver Phe hydroxylase induced by pCPA produced no

TABLE II. Free Amino Acid Concentration in the Intestinal Mucosa of Rats Under Various Diets.[a]

Diet	Intestinal mucosa free amino acid (μg/mg protein)		
	Phe	Tyr	Trp
C	8.12 ± 2.94	8.82 ± 3.65	12.78 ± 6.88
HiPhe	6.36 ± 2.11	8.78 ± 3.12	8.87 ± 3.70
pCPA	8.50 ± 1.57	10.22 ± 5.49	8.60 ± 4.62

[a] The concentration of the free amino acid in the intestinal mucosa was determined on 1:5 saline homogenates. All methods are referred to in the text. Data are means ± 95% CI. Four to 10 rats were used in each group. There was no significant difference between the C rats and the other two groups.

TABLE III. Absorption of Glucose *in Vivo* by Intestinal Segments of Rats Fed Various Diets.[a]

Group of Rats	Glucose absorption (mμmoles/min/cm)	
	20 m*M*	139 m*M*
Control	74.0 ± 15.4 (25)	488.4 ± 55.5 (4)
HiPhe	63.0 ± 29.0 (29)	380.8 ± 107.3 (4)
pCPA	75.1 ± 13.1 (11)	371.9 ± 53.9 (4)
LoPhe	73.1 ± 13.5 (16)	479.2 ± 54.8 (4)

[a] The amount of glucose transport is shown when the buffer contained 20 or 139 m*M* glucose. There were no differences in carbohydrate absorption when glucose was infused with Tyr or Trp, with or without Phe. Therefore, all the results for each diet are combined. The differences in the rate of transport by rats fed the various diets at each of both glucose concentrations were not significant. The differences in the rate of absorption achieved by animals perfused with the two concentrations of carbohydrate solutions were significant ($p < .001$). Data are means ± 95% CI. (n) = number of rats.

changes on the transport of Tyr or Trp, it could be assumed that the enzyme impairment does not cause a secondary effect at the intestinal mucosal site that could also be occurring in human PKU.

Nevertheless, untreated PKU individuals do exhibit lower plasma amino acids, other than Phe (7) and their intestinal transport of arginine, leucine and Trp (8, 9) is diminished. Therefore, other mechanisms affecting the small bowel should be considered to explain possible absorptive abnormalities in the hyperphenylalaninemic state of the PKU patients.

Summary. The local and the systemic effects of Phe on the intestinal transport of 1 m*M* Tyr and 5 m*M* Trp in Krebs–Henseleit buffers were studied *in vitro* by everted rat intestinal loops and *in vivo* by perfusion. The presence of equimolar Phe in the buffer did not inhibit the transport of Tyr and Trp in the *in vitro* everted intestinal loops, or in the *in vivo* perfusion studies. However, there was a significant inhibition in the *in vitro* and in the *in vivo* experiments (36 and 37%, respectively) when Phe was present in the buffers in a 10:1 ratio. Hyperphenylalaninemia did not alter amino acid absorption. There were no differences in the transport of Tyr and Trp both *in vitro* and *in vivo* between the intestinal loop preparations of rats fed diets containing either 1.2, 8.2 or 0.1% Phe and 0.1% pCPA.

1. Agar, W. T., Hird, F. J. R., and Sidhu, G. A., Biochim. Biophys. Acta **22**, 21 (1956).

2. Lin, E. C. C., and Wilson, T. H., Amer. J. Physiol. **199**, 127 (1960).

3. Cohen, L. L., and Huang, K. C., Amer. J. Physiol. **206**, 647 (1964).

4. Boggs, D. E., De Rapp, R. S., and McKean, C. M., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **23**, 146 (1964).

5. McKean, C. M., Boggs, D. M., and Peterson, N. A., J. Neurochem. **15**, 235 (1968).

6. Neame, K. D., Nature (London) **192**, 173 (1961).

7. Efron, M. D., Kang, E. S., Visakorp., J., and Fellers, F. X., J. Pediat. **74**, 399 (1969).

8. Linneweh, F., Ehrlich, M., Graul, E. H., and Hundeshager, H., Klin. Wochenschr. **41**, 253 (1963).

9. Yarbro, M. T., and Anderson, J. A., J. Pediat. **68**, 895 (1966).

10. Brodehl, J., Gellissen, K., and Kass, W. P., Acta Paediat. Scand. **59**, 241 (1970).

11. Wapnir, R. A., Hawkins, R. L., Stevenson, J. H., and Bessman, S. P., Biochem. Med. **3**, 397 (1970).

12. Lifshitz, F., Harrison, H. C., and Harrison, H. E., Proc. Soc. Exp. Biol. Med. **125**, 19 (1967).

13. Adibi, S. A., and Gray, S. J., Gastroenterology **52**, 837 (1967).

14. Wong, P. W. K., O'Flynn, M. E., and Inouye, T., Clin. Chem. **10**, 1098 (1964).

15. Blackburn, S., "Methods of Biochemical Analysis," Vol. 13, p. 1. Wiley (Interscience), New York (1965).

16. Wapnir, R. A., and Stevenson, J. H., Clin. Chim. Acta **26**, 203 (1969).

17. Marks, V., Clin. Chim. Acta **4**, 395 (1959).

18. Malaver, S. J., and Powell, D. W., Gastroenterology **53**, 250 (1967).

19. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

20. Natrella, M. G., "Handbook 91, National Bureau of Standards." U.S. Govt. Print. Office, Washington, DC (1965).

21. Torres-Pinedo, R., Rivera, C., and Rodriguez, H., Ann. N.Y. Acad. Sci. **176**, 284 (1971).

22. Dawson, A. M., and McMichael, H. B., J. Physiol. **196**, 32P (1968).

23. Spencer, R. P., and Samiy, A. H., Amer. J. Physiol. **199**, 1033 (1960).

24. Kerr, G. R., Chamove, A. S., Harlow, H. F., and Waisman, H. A., Pediatrics **42**, 27 (1968).

Received Nov. 24, 1971. P.S.E.B.M., 1972, Vol. 140.