PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, **147** 337-343 (1974)

The Effects of Thyroid State on Rat Liver Glucose-6-Phosphatase Activity and Glycogen Content[1] (38337)

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(Introduced by G. R. Meneely)

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Metabolically, the liver differs from most tissue in that it has the enzymatic capability to hydrolyze glucose-6-phosphate (1). This enzymatic function is of paramount importance in the control of the concentration of circulating glucose (2) upon which some tissues are dependent. The enzyme responsible for this function, glucose-6-phosphatase (G-6-Pase), is thought also to be important in the regulation of hepatocyte intracellular metabolism through its effect upon the intracellular glucose-6-phosphate (G-6-P) concentration, *e.g.*, G-6-P binds glycogen synthetase and stabilizes it in its catalytically active form, thus promoting glycogen synthesis (3, 4), and it has feedback effects on hexokinase isoenzymes and glucokinase (5, 6). The relatively uniform glucose concentration of blood is maintained presumably because hyperglycemia causes a rise in the intracellular G-6-P concentration and an increase in glycogen synthesis (2, 7). "Excess" G-6-P cannot be totally glycolyzed due to the low activity of phosphofructokinase, a rate limiting enzyme with respect to glycolysis and a metabolic bottleneck that probably controls the overall rate of glycolysis (7–10). Thus, G-6-P s thought to be directed into glycogen stores (2, 12). In contrast to hyperglycemia, hypoglycemia causes a fall in intracellular G-6-P concentration, a resultant mobilization of glycogen, and a rise in blood glucose levels (7, 11). Superimposed upon this rather autonomous process are hormonal influences such as those of insulin and cortisol.

Thyroid hormone has been shown to affect liver G-6-Pase activity and liver glycogen content markedly. The literature on this subject, however, is somewhat equivocal. Some investigators have reported greatly increased G-6-Pase activity (13–17) and decreased glycogen content (16, 18–22). Others have reported decreased G-6-Pase activity (23). There is good evidence that the response of liver and muscle to thyroid hormone is a biphasic one with low doses of the hormone (20 μg) resulting in increased glycogen synthesis, and high doses (100–200 μg) resulting in decreased glycogen synthesis (24).

A review of the literature indicates that these differences in the effects of thyroid hormone upon liver carbohydrate metabolism were in part due to: (a) the extremes of thyroid hormone dosages utilized among the various studies—from 0.3 μg to 8 mg/day administered over various periods of time, (b) different routes of administration and, sometimes, unsatisfactory solvent systems for thyroid hormone, (c) use of hypophysectomized and/or adrenalectomized animals, which are not really comparable to intact animals, even when given replacement hormones. A number of studies have been conducted that emphasize the synergistic effects of adrenal corticoids and thyroid hormone with respect to G-6-Pase activity and glycogen content (15, 25).

Only a few studies have been conducted to assess the effects of hypothyroidism *per se* upon liver G-6-Pase activity and glycogen content. Some of these studies employed the anti-thyroid agent propylthiouracil and a high carbohydrate diet. Suzuki has shown that a high carbohydrate diet of similar composition prevents the anti-thyroid effects of this drug (26). Other studies

[1] This investigation was supported in part by Public Health Service Research Grant HD-02200 from the National Institute of Child Health and Human Development.

used surgical thyroidectomy but made no provision for altered calcium metabolism due to concomitant removal of the parathyroid glands.

This study was conducted to assess the effect of extremes of thyroid states upon liver G-6-Pase activity and glycogen content. A previous study conducted under similar conditions showed that liver glucokinase activity and hexokinase isoenzyme activity were affected by thyroid state, and it was suggested that such effects might be responsible, at least in part, for the altered tolerance for glucose in thyrotoxicosis (27). The literature strongly suggests that under these conditions G-6-Pase activity would also be influenced by thyroid state. These effects in combination with those on glucokinase could be of great consequence with respect to liver carbohydrate metabolism.

Materials and Methods. Male Sprague–Dawley derived rats purchased from Charles River Breeding Laboratory, Inc. weighing 250.6 ± 9.4 g were kept two to a cage in a climate controlled room (25 ± 2° and 50–60% relative humidity) with 14 hr of light (from 4 AM to 6 PM) and 10 hr of darkness. After receiving the animals, they were maintained on Purina Lab Chow[2] and tap water (*ad libitum*) for 7 days and subsequently divided into three groups.

1. Hypothyroid group. Animals designated as hypothyroid (Hypo) were placed on an iodine deficient diet[3] and deionized water for 7 days. On day 8, radiothyroidectomies were performed by sc injection of 1.0 mCi of ^{131}I. The iodine-deficient regimen was continued for an additional 2 days, the diet was then changed to Purina Lab Chow and tap water, and daily sc injections of 0.1 ml of 2% propylene glycol in 0.9% saline (PG) were initiated.[4]

2. Euthyroid group. This group was maintained on a normal diet and tap water for the duration of the experiment. After assignment to a treatment group, they were given daily sc injections of PG. The injections were continued for 13 days.

3. Hyperthyroid group. These animals were maintained on a normal diet and tap water for the duration of the experiment. Upon assignment to a treatment group, daily sc injections of 100 μg L-thyroxine (Calbiochem) in 0.1 ml of the PG solution were administered.

General procedure. To confirm the completeness of the radiothyroidectomies all animals were given an injection of 0.1 μCi of ^{125}I on the afternoon of the day prior to sacrifice (day 13 of injections). The following morning the animals were weighed and decapitated. The entire trachea, including the larynx and thyroid gland area of each animal was removed and placed in 10% formalin solution for subsequent counting and histological processing.

Glucose-6-Phosphatase assay. Immediately after exsanguination of the animal, its liver was removed, cleaned of extraneous connective and fatty tissue, blotted and weighed. A 2.0 g portion was snipped from various sites on the liver and homogenized in ice cold 0.25 *M* sucrose (2:1 w/v) using a Potter–Elvehjem homogenizer. The volume of the homogenate was measured, and it was divided into two equal aliquots, each containing 1.0 g of liver.

G-6-Pase activity of each aliquot was immediately assayed using the technique of Nordlie and Arion (28). One unit of G-6-Pase activity was defined as that amount of activity that would hydrolyze 1 μ*M* of G-6-P to glucose and phosphate/10 min at 30°.

Liver weights and body weights of animals. The liver and body weight of each animal was recorded at the time of sacrifice. No significant differences were found between the three different treatment groups in absolute liver weight or when liver weight was expressed as percentage of body weight. Treatments had some effect on body weight. The mean body weight of the Hyper animals was 15% less ($P = < 0.05$) than that of the Eu group. The body weight of Hypo animals and Eu controls were not significantly different.

Liver glycogen content. Histological sections were made of portions of the liver of each animal, and an attempt was made to evaluate their glycogen content histochemically. The slides were numbered and arranged by the investigator in order of increasing estimated glycogen content without knowledge of the treatment groups from which they were derived. The glycogen content was then evaluated on a "+" scale of four. Those samples having very little glycogen were rated as 1+, and those with very large amounts were rated as 4+. Slides that exhibited intermediate amounts of glycogen were rated 2+ or 3+.

[2] Purina Lab Chow with a stable iodine content of 1.3–1.5 μg/g food.

[3] Remington Iodine Deficient Diet with a stable iodine content of 0.03 μg/g food.

[4] The solvent for T_4 used in the hyperthyroid animals.

It was found that those animals which had been treated with thyroxine had much less liver glycogen than either of the other groups. The rank was usually 1+ in the Hyper group, and these sections were considered glycogen depleted. It was difficult to distinguish any differences in glycogen content between the Eu and Hypo groups. The glycogen content of these two groups ranged from 3+ to 4+ (Plates 1–4).

Phosphate determination. Phosphate hydrolyzed from G-6-P by G-6-Pase was assayed using the method of Fiske and Subbarow (29) as modified by Flynn, Jones, and Lipman (30).

Histochemical evaluation of liver glycogen. An attempt to evaluate liver glycogen content morphometrically was made by removing various portions of the lobes of each liver and demonstrating hepatocyte glycogen content histochemically with malt diastase-aqueous periodic acid Schiff-hematoxylin (PAS) (31, 32). Tissue sections 5 μm in thickness were used.

Confirmation of thyroid state. Radioactivity in each thyroid gland area (removed at sacrifice) was counted in a Packard Auto-Gamma well type scintillation counter equipped with a two channel spectrometer, and the percent uptake of ^{125}I was determined. Appropriate corrections for residual ^{131}I which registered in the ^{125}I channel were made.

Serial sections were prepared from the thyroid areas of each animal and stained with hematoxylin-eosin (33–35). The functional state of the thyroid was evaluated on the basis of its histological appearance.

Results. Confirmation of thyroid state. Radioiodide uptake and histological studies confirmed each respective thyroid state. Histological examination of the thyroid glands of rats thyroidectomized with ^{131}I (Hypo group) showed only connective tissue scars with no intact follicles. These glands were nonfunctional as indicated by their inability to concentrate ^{125}I injected 18 hr previously.

Glucose-6-Phosphatase activity. Significant differences were found in liver G-6-Pase activity

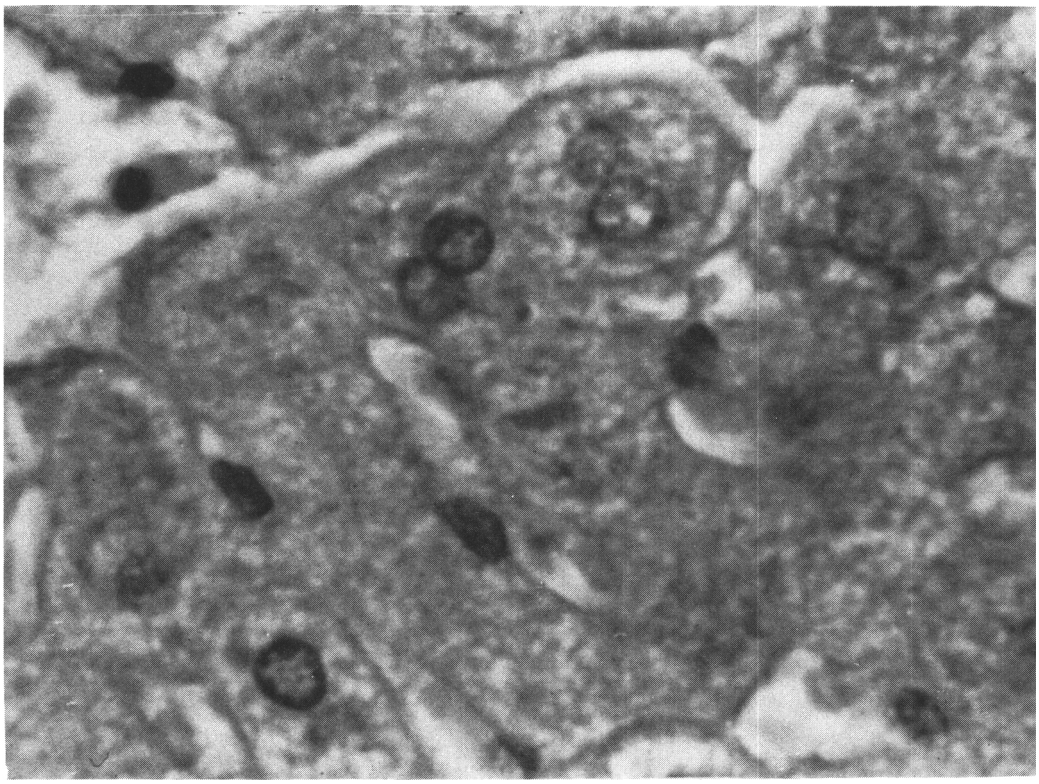

PLATE 1. PAS-diastase section of the liver of a euthyroid rat. Glycogen has been digested away by the enzyme. Photographed at × 400.

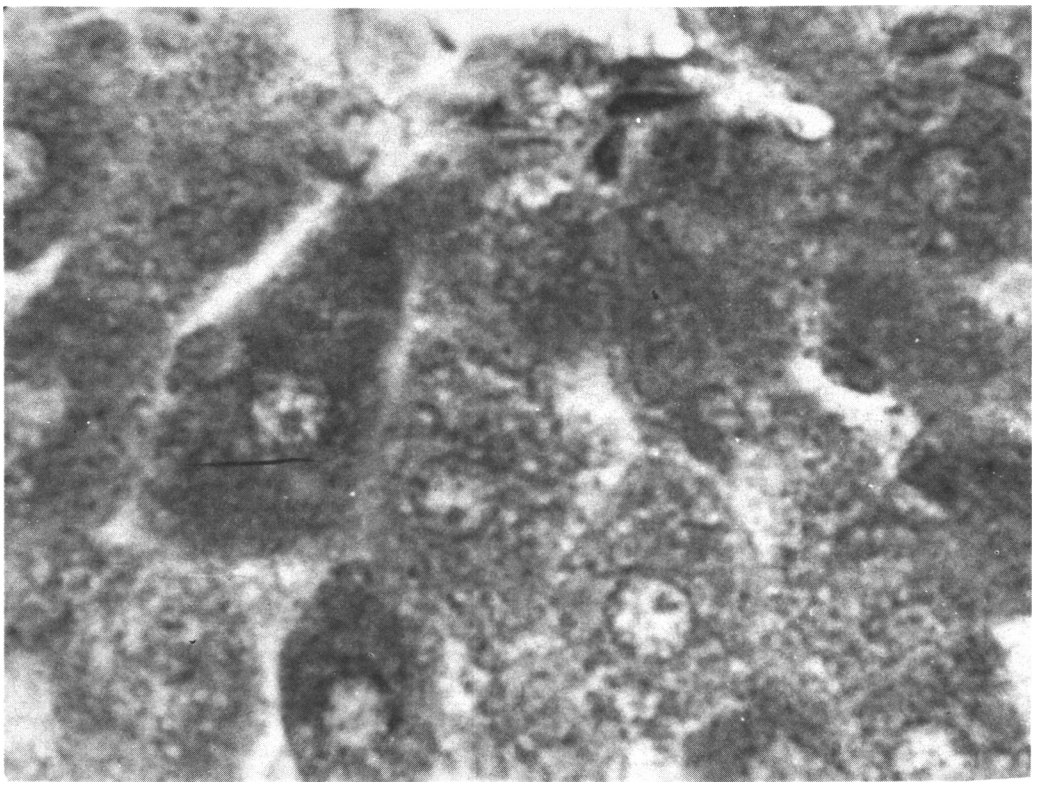

PLATE 2. PAS section of same liver lobe as in Plate 1 (Euthyroid). Glycogen remains in this section as dense staining granules. Photographed at × 400.

in different thyroid states. Mean G-6-Pase activity/g liver in the Hyper animals (350.1 ± 61.4 U/g) was increased 132% ($P = < 0.02$) over that of Eu controls (150.6 ± 17.7 U/g) and 255% ($P = < 0.01$) over that of the Hypo group (98.6 ± 16.2 U/g). No significant difference in activity was found between the Hypo and Eu animals ($P = < 0.1$) (Fig. 1).

When the activity of G-6-Pase/g body weight was compared with respect to thyroid state, G-6-Pase activity was found to be increased 134% ($P = < 0.001$) in the Hyperanimals (15.46 ± 2.58 units/g) when compared to Eu animals (6.61 ± 0.78 units/g) and increased 330% ($P = < 0.005$) when Hyper animals were compared to Hypo animals (3.59 ± 0.68 units/g). Comparison of Eu and Hypo groups revealed that G-6-Pase activity was depressed 45% in the Hypo group ($P = < 0.02$) (Fig. 1).

Expression of the data as activity/animal produced similar results. Glucose-6-phosphatase activity was found to be increased 154% ($P = < 0.02$) in the Hyper animals (3694.4 ± 692.0 units) when compared to Eu animals (1455.0 ± 174.0 units) and increased 235% ($P = < 0.01$) when Hyper animals were compared to Hypo animals (1100.9 ± 216.4 units). No significant difference was found when the activities/animal of the Eu and Hypo animals were compared (Fig. 1).

Discussion. The effects of extremes of thyroid state upon liver G-6-Pase activity and liver glycogen content was investigated in this study. Treatment of otherwise normal animals with daily sc injections of 100 μg of L-thyroxine for 13 days resulted in great increases in liver G-6-Pase activity and depleted glycogen stores. Conversely, the hypothyroid state, produced by radiothyroidectomy, resulted in glycogen stores that were indistinguishable histochemically from euthyroid control animals, and a significant reduction of G-6-Pase activity.

The preceding results indicate that in thyrotoxicosis of sufficient severity to cause a great decrease in liver glucokinase activity (23,

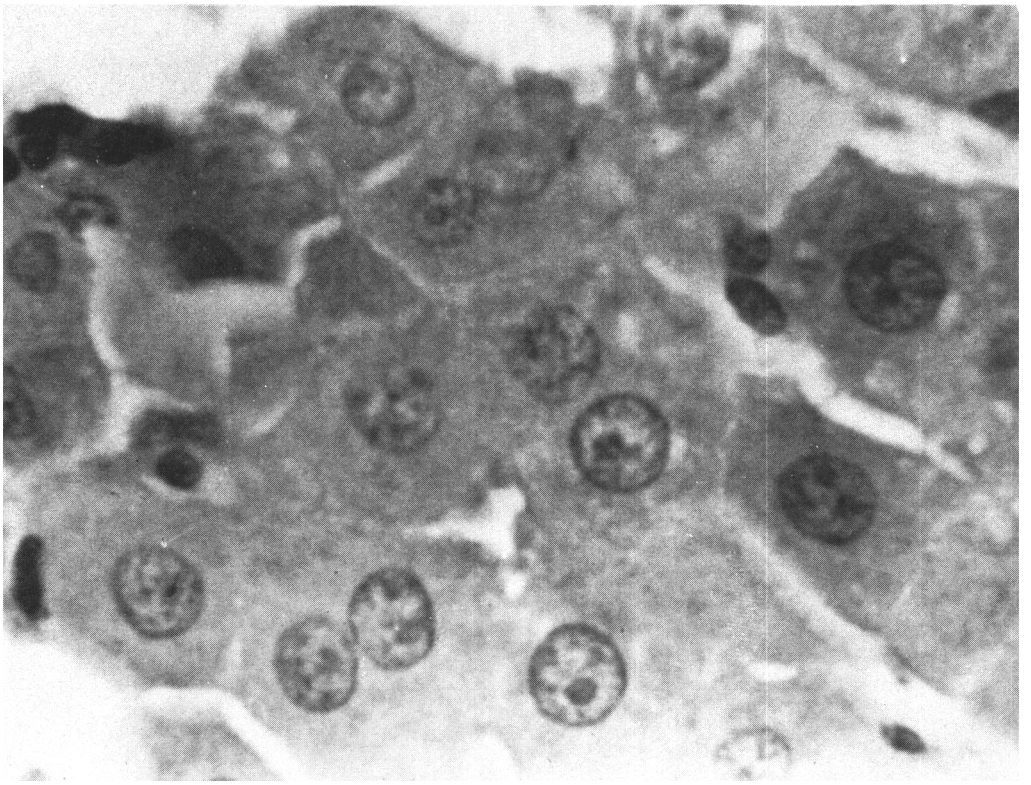

PLATE 3. PAS-diastase section of the liver of a hyperthyroid rat. Glycogen has been digested away by the enzyme. Photographed at × 400.

27), liver G-6-Pase activity is greatly increased. It has been suggested that such a change in glucokinase activity might be partly responsible for the observed changes in oral glucose tolerance curves often observed in thyrotoxicosis (27), a phenomena oftentimes ascribed to an increased absorption of glucose from the gastrointestinal tract (36). If such a reduction of glucokinase activity is accompanied by greatly increased G-6-Pase activity, the tolerance for glucose would be even further affected, especially in the postabsorptive period. Molecules of glucose would be phosphorylated by liver glucokinase at a reduced rate, and a significant number of these phosphorylated molecules would in turn be promptly dephosphorylated and returned to the systemic circulation as glucose. This would compromise the liver's ability to regulate blood sugar levels postabsorptively.

Also, since the thyrotoxic animals were all glycogen depleted and fed *ad libitum*, the data indicates that these animals were probably incapable of incurring any significant liver glycogen deposition, even in the postabsorptive state. All of the animals were sacrificed in the early morning hours after what should have been their most active feeding periods. Only the Eu and Hypo groups showed any significant stores of glycogen. This substantiates the observations of other investigators that the fraction of phosphorylated glucose that enters the liver glycogen pool is inversely proportional to liver G-6-Pase activity (2), although it must be conceded that increased glycolysis (38), pentose shunt activity and increased gluconeogenesis (37) in the thyrotoxic state may exert some effect on glycogen stores.

Summary. Treatment of animals with 100 μg of L-thyroxine resulted in great increases in liver G-6-Pase activity and depleted glycogen stores. Comparable animals rendered hypothyroid by radiothyroidectomy exhibited a decrease in G-6-Pase activity and considerable deposition of glycogen when compared to thyrotoxic animals. No differences in glycogen content were noted between euthyroid and hypothyroid animals.

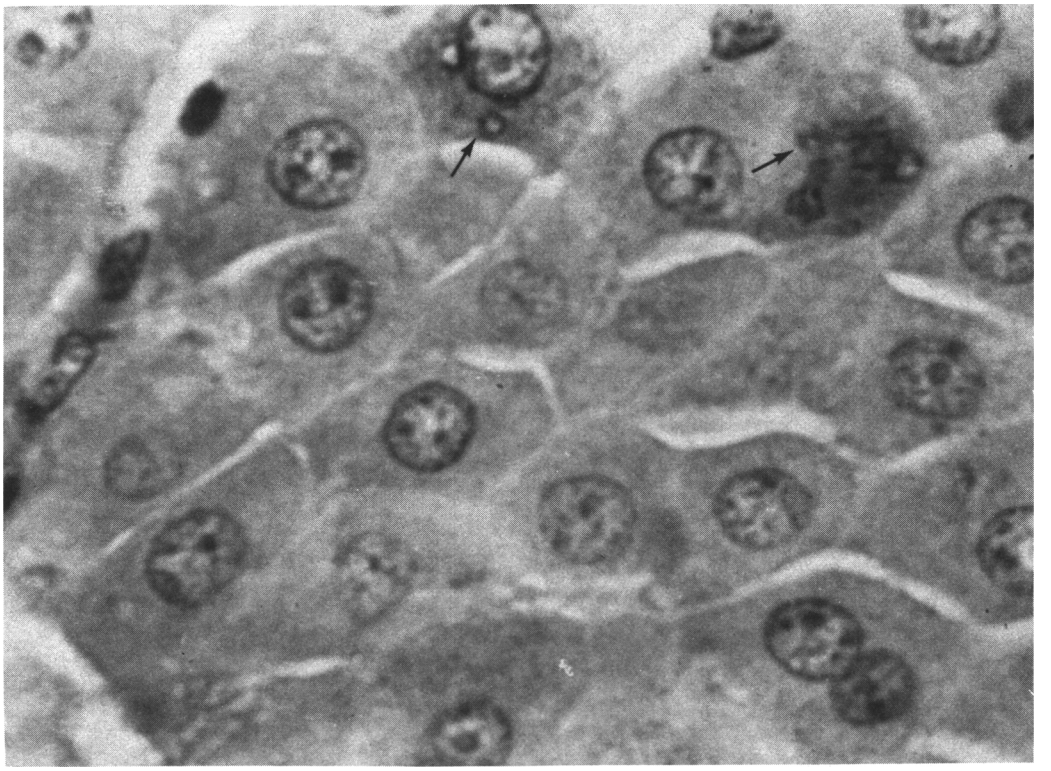

PLATE 4. PAS section of the same liver lobe as in Plate 3 (Hyperthyroid). Glycogen remains as few dense staining granules (arrows). Photographed at × 400.

Possible physiological consequences of these changes were discussed.

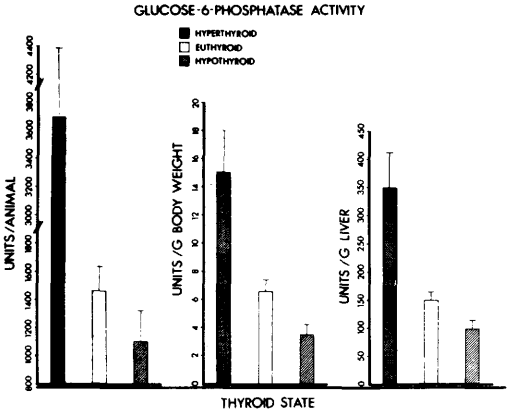

FIG. 1. Liver glucose-6-phosphatase activity in rats of various thyroid states. Bars represent group means ± SE.

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Received April 19, 1974. P.S.E.B.M., Vol. 147.