PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 166, 348–354 (1981)

Effects of Thyroid Hormone on Mitochondrial Activity in Lipemic BHE Rats (41072)[1,2]

CAROLYN D. BERDANIER AND DAVID SHUBECK

Department of Foods and Nutrition, University of Georgia, Athens, Georgia 30602

Abstract. BHE rats were treated with various levels of thyroid hormone. Their weight, relative liver size, food intake, oxygen consumption, activity of ATPase, and activity of mitochondrial shuttles were measured. It was found that the administration of thyroid hormone increased the activity of the $Ca^{2+}Mg^{2+}$ATPase and the $Na^{+}K^{+}Ca^{2+}Mg^{2+}$ATPase. The activities of the α-glycerophosphate shuttle and the state 4 of the malate–aspartate shuttle were also increased. The results indicate that the genetic aberration in metabolic control which results in maturity-onset lipemia and glycemia in BHE rats is not due to an aberration in the mitochondrial carriers of the two shuttle systems or of the ATPases.

During the last 2 years, several reports have suggested that the fundamental defect underlying the development of obesity in genetically obese rodents (ob/ob mouse, db/db mouse, Zucker rat) is a defect in the regulation of thermogenesis by thyroid hormone (1–6). Bray and York (1) found that Zucker obese rats released less thyroid hormone when given thyroid-stimulating hormone than did their lean littermates. Later (2), these same workers reported that ob/ob and db/db obese mice had lower hepatic $Na^{+}K^{+}$ATPase (EC 3.6.1.3) activities than their lean littermates but that Zucker obese and lean rats did not differ in their ATPase activity. When given daily doses of thyroid hormone, the db/db obese mouse did not show an increase in ATPase activity while the lean littermates and the obese and lean Zucker rats did (2). When ob/ob mice were treated with thyroid hormone, their ATPase activity was not increased (3); however, their lean littermates and gold thioglucose obese controls showed an increase with hormone treatment. Other workers (4) have reported a decrease in the number of [^{3}H]ouabain binding sites in muscle. In the muscles of ob/ob mouse compared to its lean littermate this decrease in the number of ouabain binding sites was interpreted as a decrease in the number of $Na^{+}K^{+}$ATPase units in this tissue (4). Another study (5) showed that there were no differences in the number of ATPase enzyme units in the liver between lean and obese littermates. While thyroid hormone treatment increased the number of ouabain binding sites in the livers of both lean and obese mice, the obese mice were two to three times more responsive to this thyroid treatment than were the lean mice. Thus, tissue differences in responsivity to thyroid hormone treatment may characterize the genetically obese rodent.

The relationship of the thyroid hormone to energy balance, food intake, and thermogenesis is far from clear. Obese mice produce more heat when starved or treated with thyroid hormone than lean mice. Yet when thyroid treated, they cannot adjust their food intake to maintain body weight so as to compensate for the thyroid-induced increase in heat production (6). The thyroid hormone regulates oxygen consumption. Obese mice consume less oxygen and gain more body fat than their lean littermates (7); however, these mice are not hypothyroid with respect to thyroid hormone levels. Thus, differences in heat production, oxygen consumption, and body fatness cannot be attributed solely to thyroid hormone level but may involve other factors such as genetically controlled differences in tissue responsiveness to the hormone.

The BHE strain of rat is derived from the same parent strain (the Osborne–Mendel) as the Zucker rat. Unlike the Zucker rat, it

[1] A preliminary report of these findings was presented at the 1979 FASEB meetings, April 1979.

[2] Supported by Georgia Experiment Station Project H635.

0037-9727/81/030348-07$01.00/0

is not obese. However, like the Zucker, it has a higher than normal hepatic lipogenic capacity (8–15), a disordered insulin–glucose relationship (10), and a maturity-onset carbohydrate-induced lipemia/glycemia (8, 9, 14, 15). The BHE rat appears to have an increase in hepatic cytosolic lipogenesis at the expense of oxidative phosphorylation (13–15). Studies of mitochondrial activity showed a decrease in oxygen uptake (respiration), a decrease in α-glycerophosphate shuttle activity, a decrease in the state 4-malate–aspartate shuttle activity, and an increase in cytosolic and mitochondrial phosphorylation states (13–15) compared to a nonlipemic strain of rats (Wistar). The activities of the various ATPases of the mitochondria and microsomes have been determined and found to be in the normal range. Since thyroid hormone (T_4) has been reported to have a regulatory function with respect to phosphorylation state as well as shuttle activities and mitochondrial respiration (16, 17) and because of the reports of an altered sensitivity to this hormone by obese rodents (1–7), we sought to determine whether this hormone would increase hepatic mitochondrial activity in BHE rats. We found that administration of the hormone increased the activities of the $Ca^{2+}Mg^{2+}$ATPase, the α-glycerophosphate shuttle and the state 4-malate–aspartate shuttle.

Materials and methods. Four groups of eight male 50-day-old inbred (line IV) BHE rats were selected from the BHE colony maintained at the University of Georgia. The rats were housed individually in wire-mesh-hanging cages in a temperature-humidity controlled room having equal 12-hr periods of light and dark (lights on, 0600). Food[3] and water were always available. Body weight and food intake were determined daily. One group of rats served as the control and was injected twice daily with isotonic saline. The other groups were injected, ip, with 10, 20, or 40 μg T_4/100 g body wt divided into two doses administered at 12-hr intervals. Thyroxine was dissolved in 5 m*M* NaOH to a concentration of 1 mg/ml. The solution was made fresh daily. After 2–3 days of treatment, the oxygen consumption[4] of the animals was measured daily to determine the effectiveness of the T_4 treatment.

After 7 days of treatment, the animals were decapitated, the livers excised, chilled, weighed, and used for the determination of mitochondrial ATPase and shuttle activities. The mitochondria were prepared according to the procedures of Johnson and Lardy (18). Shuttle activities were determined, as previously described (13), in the presence or absence of 4 m*M* ADP to simulate state 3 or state 4 respiratory conditions.[5] ATPase (ATP phosphohydrolase, EC 3.6.1.3) activity of 1 mg mitochondrial protein was determined in a medium containing 39 m*M* Tris (pH 7.4), 3 m*M* Mg^{2+} (from $MgCl_2$), 0.5 m*M* Ca^{2+} (from $CaCl_2$), 3 m*M* ATP, 100 m*M* Na^+ (from NaCl), and 30 m*M* K^+ (from KCl). Ca^{2+}, Na^+, and K^+ were omitted from the medium when Mg^{2+} ATPase activity was determined; Na^+ and K^+ were omitted when $Ca^{2+}Mg^{2+}$ATPase was determined. Glycine was added to maintain osmolarity. In previous studies, mannitol was used as the osmotic agent (19); however, we subsequently learned that this compound, in high levels, inhibited (*in vitro*) the activity of the ATPases. While having no effect on the ATPase measured in the presence of all four salts, when only one or two of the salts were used (so as to measure the Mg-stimulable or Ca-stimulable), mannitol, because of its higher concentration in these cocktails, inhibited P_i release. The ATPase activities were determined in the presence or absence of 10^{-3} ouabain. Mitochondrial protein was determined by biuret. ATPase activity was measured as the amount of inorganic phosphate released per milligram of protein per hour. Inorganic phosphate was determined using the Harris and Popat (20) modification of the Fiske and Subbarow method (21). Significant

[3] Purina Laboratory Animal Chow, Ralston Purina Company.

[4] Whole body respirimeter.

[5] State 3 is the condition of mitochondrial respiration in the presence of ADP, substrate, inorganic phosphate, and other factors necessary for maximal coupled respiration. State 4 is the condition after ADP has been phosphorylated to ATP.

TABLE I. EFFECT OF THYROID HORMONE TREATMENT ON BODY WEIGHTS, FOOD INTAKES, RELATIVE LIVER SIZE, AND OXYGEN CONSUMPTION OF BHE RATS

Treatment	Initial weight (g)	Final weight (g)	RLS[a]	Food intake (g)	Oxygen consumption (ml/kg/24 hr)
Control	199 ± 8[b]	250 ± 8	4.34 ± 0.14	25 ± 1	56 ± 2
10 μg T_4/100 g body wt	196 ± 9	243 ± 10	4.33 ± 0.14	25 ± 1	69 ± 3[c]
20 μg T_4/100 g body wt	181 ± 7	226 ± 7[c]	4.02 ± 0.04[c]	22 ± 1	74 ± 2[c]
40 μg T_4/100 g body wt	190 ± 13	229 ± 13[c]	4.09 ± 0.08[c]	24 ± 1	72 ± 3[c]

[a] RLS, relative liver size = liver weight/body weight × 100.
[b] Mean ± SEM for eight rats.
[c] Effect of thyroid treatment is significant ($P < 0.05$).

differences between control and T_4-treated groups were determined using the Student *t* test.

Results. Mean initial weights of the 50-day-old rats used in this experiment ranged from 181 to 199 g (Table I). Treatment with 10 μg T_4/100 g body wt for 7 days did not affect the weight gain of these rats compared to that of the control rats but treatment with higher levels of the hormone did. The final body weights of rats given 20 or 40 μg T_4/100 g body wt/day were significantly less than those of the control rats. Relative liver size (RLS) followed this same pattern. There were no differences in food intake, and, as expected, oxygen consumption rose with the level of thyroid treatment.

The activities of the ATPase are presented in Table II. T_4 treatment did not affect the Mg^{2+} ATPase, the enzyme which catalyzes the slow synthesis of ATP from ADP and P_i. Ouabain does not affect the activity of this ATPase. Similarly, ouabain did not affect the $Ca^{2+}Mg^{2+}$ATPase, but this ATPase was increased in rats treated with 10 μg T_4/100 g/day; no further increases in the activity of this enzyme were observed when greater amounts of thyroid hormone were injected. This is consistent with our previous report using Sprague–Dawley rats (19). This was also true for the ATPase measured in the presence of all four ions with or without ouabain. However, based on the observed increase in the $Ca^{2+}Mg^{2+}$ATPase with thyroid hormone treatment, the increase in ATPase activity when all four ions were present was probably due to the increase in $Ca^{2+}Mg^{2+}$ATPase rather than to any increase in the Na^+K^+-ATPase. Again, this is consistent with our previous report (19). What is not consistent with our previous report is the effect of

TABLE II. EFFECT OF THYROID DOSE ON HEPATIC MITOCHONDRIAL ATPASE ACTIVITY

ATPase	Ouabain addition	Control	T_4 dose (μg/100 g body wt/day) 10	20	40
			μg P_i/mg protein/hr		
Mg^{2+}[a]	−	0.82 ± 0.41[b]	1.46 ± 0.62	2.57 ± 1.25	0.62 ± 1.87
	+	1.65 ± 0.82	1.62 ± 0.48	2.85 ± 1.40	2.72 ± 1.39
$Ca^{2+}Mg^{2+}$[a]	−	15.87 ± 1.42	26.13 ± 2.63[c]	22.39 ± 1.11[c]	24.0 ± 2.75[c]
	+	18.81 ± 4.88	21.12 ± 2.03[c]	20.67 ± 0.99[c]	21.0 ± 2.80[c]
	−	14.31 ± 1.31	23.22 ± 1.09[c]	21.81 ± 1.44[c]	25.17 ± 3.13[c]
$Na^+K^+Ca^{2+}Mg^{2+}$[a]	+	9.00 ± 0.79[d]	16.41 ± 0.80[c,d]	17.01 ± 1.28[c,d]	18.81 ± 2.47[c]

[a] ATPase activity was determined in a medium containing 39 m*M* Tris (pH 7.4), 3 m*M* Mg^{2+} (from $MgCl_2$), 0.5 m*M* Ca^{2+} (from $CaCl_2$), 3 m*M* ATP, 100 m*M* Na^+ (from NaCl), 30 m*M* K^+ (from KCl). Na^+ and K^+ were omitted from medium when $Ca^{2+}Mg^{2+}$ activated ATPase was determined. Ca^{2+}, Na^+, and K^+ were omitted when Mg^{2+} ATPase activity was determined. Glycine was added to maintain osmolarity.
[b] Mean ± SEM eight rats/group.
[c] Effect of thyroid treatment was significant ($P < 0.05$).
[d] Effect of ouabain addition was significant ($P < 0.05$).

ouabain addition to the cocktail containing all four ions. In the present report, ouabain addition appeared to decrease the activity of the ATPase measured in the presence of all four ions. Since ouabain not only inhibits Na^+K^+ATPase but also mitochondrial oxygen consumption, its effect on ATPase activity in a situation where the Na^+K^+-ATPase's activity is negligible is puzzling unless, of course, one assumes that in this situation ouabain's action is on calcium and magnesium flux. Both of these ions in the presence of Na^+ and K^+ have been shown to be involved in the regulation of mitochondrial activity (22–28). Calcium and magnesium ion movements across the mitochondrial membrane are interdependent and both are influenced by thyroid hormone (22, 26, 27). Calcium ion flux strongly influences ADP/ATP exchange which regulates, in part, mitochondrial respiration (25–31). Thus, an effect of ouabain on mitochondrial oxygen consumption may in fact relate to its effect on the $Ca^{2+}Mg^{2+}$ flux. Further studies are needed to support this hypothesis.

The influence of T_4 treatment on mitochondrial shuttle activities is presented in Table III. Endogenous NAD^+ production by isolated mitochondria in the absence of added ADP increased slightly with increasing levels of T_4 treatment. In the presence of ADP, this endogenous NAD^+ production was lower than in the absence of ADP, and 10 or 20 μg T_4 treatment had no effect. Malate–aspartate shuttle activity in the absence of added ADP was greater in T_4-treated rats than in the control rats, but T_4 treatment did not affect the activity of this shuttle under state 3 conditions. Malate–aspartate shuttle activity determined as NAD^+ production by mitochondria from the control rats in the presence or absence of ADP was similar to that reported previously (13). Added ADP slightly stimulated malate–aspartate shuttle activity above that observed in mitochondria incubated without added ADP. In T_4-treated rats, the reverse was observed. ADP addition resulted in a decrease in NAD^+ production.

As expected, T_4 treatment resulted in a marked increase in the activity of the α-glycerophosphate shuttle probably due to an effect of the hormone on the mitochondrial α-glycerophosphate dehydrogenase activity (16, 17, 32). BHE rats have been shown to have a less active enzmye (12) than Wistar rats, and the T_4 treatment probably increased the synthesis of this enzyme. The observations of the activity of this shuttle in the control rats are consistent with those reported previously (12, 13). When the activity of this shuttle was determined in the presence of ADP, there was an increase in activity as the level of T_4 treatment increased. Although the α-glycerophosphate shuttle is not considered to be controlled by the ADP, it is apparent that under the conditions of the present study, ADP presence and T_4 treatment interacted and resulted in an increase in α-glycerophosphate shuttle activity.

Discussion. In earlier studies, we showed that elevated redox states characterized but did not control the lipogenic capacities of BHE rats (13–15). In young rats, we observed both raised redox states and increases in lipogenesis (9–13). However, as BHE rats aged, the redox ratios declined, yet, their lipogenic capacities remained high (14, 15). These observations lead to the conclusion that NAD^+/NADH and $NADP^+$/NADPH ratios were not rate limiting with respect to lipogenesis. This conclusion then poses the question as to what other cellular characteristics have control properties with respect to lipogenesis in these rats. A priori there most be adequate communication between cellular compartments vis a vis metabolite exchange, adenine nucleotide transloction and anion/cation exchange. If there is any hindrance in any or all of these processes, then one might anticipate profound changes in the fuel economy of the cell. All of these systems appear to be influenced by thyroid hormone. As shown by the present work, T_4 increases the activities of the Ca^{2+} Mg^{2+} ATPase and the α-glycerophosphate shuttle. At the low dosage level, T_4 did not affect either body weight gain or food intake but increased oxygen consumption. What was unique in this work was the finding that T_4 "normalized" the malate–aspartate shuttle in the BHE rat. In our previous re-

TABLE III. EFFECT OF THYROID DOSE ON THE SHUTTLE ACTIVITIES OF ISOLATED HEPATIC MITOCHONDRIA FROM YOUNG BHE MALE RATS

Shuttle	4 m*M* ADP addition	Control	T_4 Dose μg/100 g body wt/day		
			10	20	40
			(μmole NAD^+ produced/mg protein/min)		
Endogenous[a]	−	10.08 ± 0.99[b]	12.17 ± 1.10	13.39 ± 1.18[f]	13.75 ± 1.39[f]
	+	3.99 ± 1.03[c]	5.37 ± 0.95[c]	5.61 ± 0.96[c,f]	6.36 ± 0.91[c]
Malate−aspartate[d]	−	15.02 ± 0.21[e]	27.52 ± 1.55[e,f]	26.58 ± 1.90[e,f]	26.68 ± 2.07[e,f]
	+	19.42 ± 1.31[e]	16.68 ± 1.56[c,e]	22.22 ± 4.55[e,h]	19.19 ± 1.81[c,e]
α-Glycerophosphate[g]	−	9.64 ± 1.11	45.46 ± 3.15[f,h]	41.23 ± 5.22[f,h]	51.03 ± 2.07[f,h]
	+	7.33 ± 1.44	49.98 ± 4.19[f,h]	56.0 ± 4.0[c,f,h]	62.01 ± 5.31[c,f,h]

[a] Five milligrams of mitochondrial protein were suspended in a medium containing 75 m*M* glycine, 10 m*M* KH_2PO_4 (pH 7.4) 5 m*M* $MgCl_2$, 75 m*M* KCl, 10 m*M* Tris HCl (pH 7.4), 4 m*M* ADP as indicated, 5 m*M* NADH, and water to a volume of 3 ml. Flasks were incubated for 20 min at 30°.

[b] Mean ± SEM eight rats/group.

[c] Effect of addition of ADP is significant ($P < 0.05$).

[d] The complete system contained, in addition to the above, 1 m*M* malate, 5 m*M* glutamate, 2 m*M* aspartate, 29 units of malate dehydrogenase, 25 units of glutamic oxaloacetic transaminase, and water to a volume of 3 ml.

[e] Effect of addition of malate−aspartate shuttle components is significant ($P < 0.05$).

[f] Effect of thyroid hormone treatment is significant ($P < 0.05$).

[g] The complete system contained, in addition to the ingredients listed in footnote *a*, 20 m*M* DL-α-glycerophosphate, 5 units of α-glycerophosphate dehydrogenase, and 30 nmole rotenone/g protein in a final volume of 3 ml.

[h] The effect of the addition of α-glycerophosphate shuttle components is significant ($P < 0.05$).

port (13), the malate–aspartate shuttle in Wistar mitochondria was less active when ADP was added to the incubation; in BHE mitochondria, the reverse was true, suggesting that *in vivo* ADP may have been limited. ADP concentration *in vivo* is known to control the activity of this shuttle (33–36). That ADP may have been limited was seen in the raised phosphorylation states (higher levels of ATP relative to ADP) of these rats compared to the Wistar rats. In the present study, mitochondria from T_4-treated BHE rats were less active in simulated state 3 conditions than in state 4 conditions suggesting that treatment with T_4 relieved the *in vivo* limitation on ADP availability. Studies on the effects of T_4 on phosphorylation state have shown that treatment with T_4 lowers phosphorylation state in frozen clamped livers from Sprague–Dawley rats (37). No doubt this has also occurred with T_4 treatment in the present study. Whether the decrease in phosphorylation state was due solely to increase in the activities of the ATPases with T_4 treatment is doubtful. More likely, this T_4 effect can be explained as being due both to an increase in the synthesis and/or activation of adenine nucleotide translocase (27, 38) and an increase in ATPase activities. With respect to the translocase, this carrier protein is inhibited by long-chain fatty acyl-CoA esters. Perhaps the BHE rats, due to their high lipogenic capacity, produce sufficient fatty acids to inhibit translocase activity. When treated with T_4, these rats may increase their oxidation of these fatty acids thereby relieving the translocase inhibition. Alternatively, T_4 treatment may simply have increased the synthesis of the translocase just as it influences the synthesis of a number of other cellular proteins. In fact, Weiss and Sokoloff (39) as early as 1963 suggested that the larger fraction of the total body basal oxygen consumption in hyperthyroidism is related to the process of protein synthesis rather than to the increase in mitochondrial respiration *per se*.

Although BHE rats have not been found to be hypothyroid in terms of oxygen consumption or serum hormone levels,[6] it is possible that there may be, *in vivo*, some slight decrease in the responsiveness of the BHE tissues to the hormone which could explain the present results. Clearly, the genetic aberration in metabolic control which results in the maturity-onset lipemia and glycemia is not due to a genetic aberration in the structure of either of the mitochondrial carriers of the two shuttle systems or of the ATPases studied here. If there were genetically determined differences in these proteins, we would not have been able to induce their activity with T_4 treatment. Thus, we have eliminated one more possible locus of a genetic error which conceivably could be responsible for the characteristic traits of this strain.

[6] Serum T_4 values for Sprague–Dawley, Wistar, and BHE rats have been measured as 2.0 ± 0.8.

The authors would like to thank Ms. Cindy Wolfe and Ms. Jean Stevens for their excellent technical assistance.

1. Bray, G. A., and York D. A., Endocrinology, **88**, 1095 (1971).
2. Bray, G. A., York, D. A., and Yukimura, Y., Life Sci. **22**, 1637 (1978).
3. York, D. A., Bray, G. A., and Yukimura, Y., Proc. Nat. Acad. Sci. USA, **75**, 477 (1978).
4. Lin, M. H., Romsos, D. R., Akera, T., and Leveille, G. A., Biochem. Biophys. Res. Commun. **80**, 398 (1978).
5. Lin, M. H., Vander Tuig, J. G., Romsos, D. R., Akera, T., and Leveille, G. A., Amer. J. Physiol. **237**, 265 (1979).
6. Vander Tuig, J. G., Trostler, N., Romsos, D. R., and Leveille, G. A., Proc. Soc. Exp. Biol. Med. **160**, 266 (1979).
7. Boissonneault, G. A., Hornshuh, M. J., Simons, J. W., Romsos, D. R., and Leveille, G. A., Proc. Soc. Exp. Biol. Med. **157**, 402 (1978).
8. Berdanier, C. D., J. Nutr. **104**, 1246 (1974).
9. Berdanier, C. D., Diabetologia **10**, 691 (1974).
10. Berdanier, C. D., Fed. Proc. **35**, 2295 (1976).
11. Lakshmanan, M. K., Berdanier, C. D., and Veech, R. L., Arch. Biochim. Biophys. **183**, 355 (1977).
12. Berdanier, C. D., Tobin, R. B., DeVore, V., and Wurdeman, R., Proc. Soc. Exp. Biol. Med. **157**, 5 (1978).
13. Berdanier, C. D., Tobin, R. B., and DeVore, V., J. Nutr. **109**, 247 (1979).
14. Berdanier, C. D., Tobin, R. B., and DeVore, V., J. Nutr. **109**, 261 (1979).
15. Berdanier, C. D., Tobin, R. B., DeVore, V., and Cook, L., J. Nutr. **109**, 272 (1979).
16. Meijer, A. J., and VanDam, K., Biochim. Biophys. Acta **346**, 213 (1974).

17. Sterling, K., N. Engl. J. Med. **300**, 117 and 173 (1979).
18. Johnson, D., and Lardy, H., *in* "Methods in Enzymology" (R. W. Estabrook and M. E. Pullman, eds.), Vol. 10, p. 94. Academic Press, New York (1967).
19. Tobin, R. B., Berdanier, C. D., and Eckland, R. E., J. Environ. Pathol. Toxicol. **2**, 1247 (1979).
20. Harris, W. D., and Popat, P., J. Amer. Oil Chem. Soc. **32**, 241 (1954).
21. Fiske, C. H., and Subbarow, Y., J. Biol. Chem. **66**, 375 (1925).
22. Becker, G. L., Biochem. Biophys. Acta **591**, 234 (1980).
23. Nichols, D. G., and Crompton, M., FEBS Lett. **111**, 261 (1980).
24. Froehlich, J. P., and Taylor, E. W., J. Biol. Chem. **251**, 2302 (1976).
25. Matlib, M. A., Wilson, D., Rouslin, W., Kraft, G., Bemer, P., and Schartz, A., Biochem. Biophys. Res. Commun. **84**, 482 (1978).
26. Herd, P. A., Arch. Biochem. Biophys. **188**, 220 (1978).
27. Hoch, F. L., Arch. Biochem. Biophys. **178**, 535 (1977).
28. Kimura, S., and Rasmussen, H., J. Biol. Chem. **252**, 1217 (1977).
29. Hunter, D. R., and Haworth, R. A., Arch. Biochem. Biophys. **195**, 453 (1979).
30. Fiskum, G., and Lehninger, A. L., J. Biol. Chem. **254**, 6236 (1979).
31. Fiskum, G., Roynafarje, B., and Lehninger, A. L., J. Biol. Chem. **254**, 6288 (1979).

Received March 27, 1980. P.S.E.B.M. 1981, Vol. 166.