PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 167, 506–513 (1981)

Elevated Arterial Pressure, Vascular Wall "Waterlogging," and Impaired Cardiac Growth in Rats Chronically Receiving Digoxin[1] (41205)

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Abstract. Decreased activity of the sarcolemmal sodium pump may account, in part, for the elevated arterial pressure and also for the vascular wall "waterlogging" in certain forms of hypertension. To further test this hypothesis, digoxin, 120 or 240 mg/kg/day, was administered orally to 6-week-old rats and continued for 6–7 weeks in Group A rats. After 5 weeks of similar administration, digoxin was stopped in Group B rats. Group C control rats never received digoxin. Measured serum digoxin levels in Group A rats ranged from 204 to 660 ng/ml. In rats receiving digoxin, tail systolic arterial pressures measured three times per week were slightly (6%) but significantly ($P < 0.001$) higher than in control rats. At age 12 weeks, tail systolic pressures (mm Hg; M ± SEM) were 128.2 ± 1.1, 118.4 ± 0.7, and 118.0 ± 0.3 in Groups A, B, and C. At age 12–13 weeks, water content of the thoracic vena cava in digoxin-treated rats of Group A was increased by 3–5% ($P < 0.05$). An additional finding was that left ventricular weight and LV weight/body wt in Group A rats were decreased by 4–9% ($P < 0.05$). These data indicate that, in rats, chronic inhibition of the membrane sodium pump in the cardiovascular muscle by digitalis is associated with increased arterial pressure and vascular wall waterlogging. Thus, pump inhibition may play a role in similar abnormalities occurring in hypertension. Digoxin administration also impaired cardiac growth in these young rats.

We recently reported (1) that the chronic (4 weeks') administration of digoxin to dogs is accompanied by suppression of sodium pump activity in vascular smooth muscle and "waterlogging" of vascular walls. Similar abnormalities occur in certain forms of hypertension (2–5). These findings were compatible with our hypothesis (3) that inhibition of the sarcolemmal sodium pump of vessels in hypertension may underlie their waterlogging. However, these dogs did not develop the hypertension we anticipated (2, 3), even though the peripheral resistance was probably elevated (6, 7), and even though hypertension with acute digitalis administration has been documented (6, 7). Failure of our dogs to develop hypertension may have been species related or due to decreases in body fluid volumes and, hence, cardiac output, accompanying the digoxin treatment. Alternatively, the techniques we used to measure blood pressure (weekly femoral arterial punctures) may not have been sufficiently sensitive to allow us to detect small elevations in pressure. Therefore, the purpose of the present study was to repeat these experiments by administering digoxin for several weeks to rats, a species in which multiple measurements of arterial pressure may be easily obtained and small changes in blood pressure detected.

Materials and Methods. *Experiment 1.* Male Sprague–Dawley rats, age 6 weeks, body weights 198 ± 6 g (M ± SEM), were randomly divided into three groups. Group C rats ($N = 5$), the control group, received normal rat chow (Na^+ 0.39%, K^+ 0.96%). Group A rats ($N = 10$) received 120 mg digoxin/kg/day (pulverized Lanoxin tabs; Burroughs Wellcome Co.) mixed with their chow for 6–7 weeks. Group B rats ($N = 9$) received digoxin identically for 5 weeks and then were given only normal rat chow for a final 1–2 weeks. All rats drank water *ad libitum*. Body weights and conscious tail systolic blood pressures by the cuff method (Natsume Tail Manometer System) were

[1] This study was supported by U.S. Public Health Service Research Grants HL-23600 and HL-23312. Portions of this work were published in abstract form (*Clin. Res.* 27, 675A, 1979).

0037-9727/81/080506-08$01.00/0

measured every Monday, Wednesday, and Friday in all rats. At age 12–13 weeks the rats were killed by decapitation, serum was obtained for measurements of concentration of digoxin (Digoxin RIA Kit, Beckman Instruments, Inc., Irvine, Calif.) and of K^+, Na^+, and Ca^{2+} (flame photometer). The left ventricle (including septum), a standardized segment of the thoracic portion of the inferior vena cava, and, in some rats, a standardized segment of the thoracic aorta, were obtained for measurement of weight and wall water content by techniques we have previously described (8). These techniques call for reweighing after 24 hr at 100°, at which time we have documented a stable dry weight.

We additionally measured the total and ouabain-insensitive components of ^{86}Rb uptake by aorta freshly excised from some of these rats; we used techniques similar to those we reported for dogs (1) and rats (9) (see also below, under Experiment 3).

Experiment 2. An additional 34 male Sprague–Dawley 6-week-old rats, body weights 202 ± 2 g, were randomly divided into Groups A (N = 11), B (N = 11), and C (N = 12), and subjected to a protocol identical to that of Experiment 1 with the following exceptions: (i) the Group A and B rats received 240 mg/kg/day of pure digoxin powder (Lanoxin, Burroughs Wellcome Co.) mixed with chow; (ii) blood pressures were measured for only the first 2 weeks of digoxin administration; and (iii) the right ventricle was also obtained for weight and water content. Because no significant differences were found in weights and water contents of tissues from rats of Experiment 2 as compared to Experiment 1, these values were pooled for data analysis. Group comparisons were by analysis of variance followed, if significant, by Newman–Keuls and/or Student's t test (10).

Experiment 3. In an additional 16 male Sprague–Dawley rats, body weights approximately 400 g, we used methods similar to those we have previously described (9) to investigate the relationship between digitalis concentration and sodium pump activity in rat vascular smooth muscle. Because higher concentrations of digoxin are relatively insoluble in aqueous solutions, we used ouabain to describe the relationship. However, we also tested two levels of digoxin for comparison. Each rat was anesthetized with pentobarbital (60 mg/kg, ip). The descending thoracic aorta was gently and rapidly excised, cleaned of adventitia and blood, and opened longitudinally. Each aorta was divided into four equal segments which were pooled and stored overnight in the refrigerator at 4° in Krebs–Henseleit solution ($NaHCO_3$, 27.2 m*M*; NaCl, 118.0 m*M*; KH_2PO_4, 1.0 m*M*; KCl, 4.8 m*M*; $MgSO_4 \cdot 7H_2O$, 1.2 mM; $CaCl_2 \cdot 2H_2O$, 1.25 mM; and glucose, 11.1 mM). The following morning, to restore intracellular ion contents toward normal levels, these segments were incubated for 3 hr at 37° in Krebs–Henseleit solution bubbled with 95% O_2, 5% CO_2 (pH 7.4). Next the segments were incubated for 10 min at 37° in O_2–CO_2-bubbled Krebs–Henseleit solution containing trace amounts of $^{86}RbCl$ (New England Nuclear) and varying amounts of ouabain or digoxin. Six segments were incubated at each of the following ouabain concentrations: 0 m*M*; 0.0612 m*M*; 0.25 m*M*; 0.5 m*M*; 1.0 m*M*; and 2.0 m*M*. Twelve segments were incubated at 0.125 m*M* ouabain. Six segments were incubated at each of the following digoxin concentrations: 0.25 and 10 μM. Tissues were then washed three times (total time 15–20 sec) with 0° Krebs–Henseleit, blotted with tissue paper to remove surface fluid, weighed, and placed in a crystal scintillation counter to determine ^{86}Rb uptake. ^{86}Rb uptake was calculated as nanomoles per milligram of wet weight/10 min. Total ^{86}Rb uptake was plotted against ouabain concentration.

Results. Terminal serum digoxin concentrations (ng/ml, M ± SEM and range) were Group A (N = 19), 299.4 ± 22.1, 204–660; Group B (N = 17), 10.6 ± 5.6, 1–96. Concentrations in Group C on normal chow were not measured. Terminal serum K^+ (mEq/liter) were Group A (N = 8) 3.40 ± 0.11 and Group B (N = 7) 3.63 ± 0.17. Terminal serum Na^+ (mEq/liter) were Group A (N = 11) 138.6 ± 1.3 and Group B (N = 10) 138.0 ± 0.8. Terminal serum Ca^{2+} (mEq/liter) were Group A (N = 11) 2.88 ± 0.04 and Group B (N = 10) 2.94 ± 0.04.

These values for serum electrolytes were not significantly different among the groups.

The rats appeared healthy during the experimental period. Figure 1 presents body weights and tail systolic blood pressures (M ± SEM) plotted against time in rats of Experiment 1. As would be expected, weights and blood pressures in Groups A and B did not differ through the fifth week of digoxin administration and were pooled. A persistent growth defect appearing 3 days after starting digoxin is illustrated in the figure. At the third week of digoxin administration, for example, body weights (g; M ± SEM) in the three groups were: Group A (N = 10) 288.4 ± 6.6, Group B (N = 9) 273 ± 5.9, and Group C (N = 5) 328.4 ± 13.1. Analysis of variance revealed significant differences ($P < 0.005$) among the groups. Groups A and B were not different ($P > 0.25$), but body weights of Group C were greater than those of either Group A or B rats by Newman–Keuls. Shortly before sacrifice, at the sixth week, body weights of the three groups were not significantly different: Group A (N = 10) 354.2 ± 9.6; Group B (N = 9) 337.3 ± 7.8; Group C (N = 5) 397.2 ± 29.7.

Figure 1 also illustrates the small persistent elevation in arterial pressure occuring within 1 week after start of digoxin treatment. Comparison of systolic pressure in each rat before and during the first week of digoxin by Student's t test for paired replicates revealed that the increases in rats of Groups A and B, receiving digoxin, were highly significant ($P < 0.001$), whereas no significant change occurred in Group C control rats ($P > 0.2$). At the third week of digoxin administration, systolic blood pressures (mm Hg; M ± SEM) in the three groups were: Group A (N = 10) 129.1 ± 1.6; Group B (N = 9) 124.6 ± 2.0; and Group C (N = 5) 118.2 ± 2.2. Analysis of variance revealed significant differences ($P < 0.01$) among the groups. By Newman–Keuls test, Groups A and B were not different ($P > 0.1$), whereas pressures in Group C rats were lower than those in both Groups A and B. In Group B rats, the elevated pressures disappeared within three days after digoxin was stopped ($P < 0.001$ by paired Student's t test). Shortly before sacrifice, at the sixth week, blood pressures in the three groups were: Group A (N = 10) 128.2 ± 1.1; Group B (N = 9) 118.4 ± 0.7; Group C (N = 5) 118.0 ± 0.3. Analysis of variance again indicated significant differences ($P < 0.001$) among the groups. Newman–Keuls test indicated no difference between Groups B and C, whereas blood

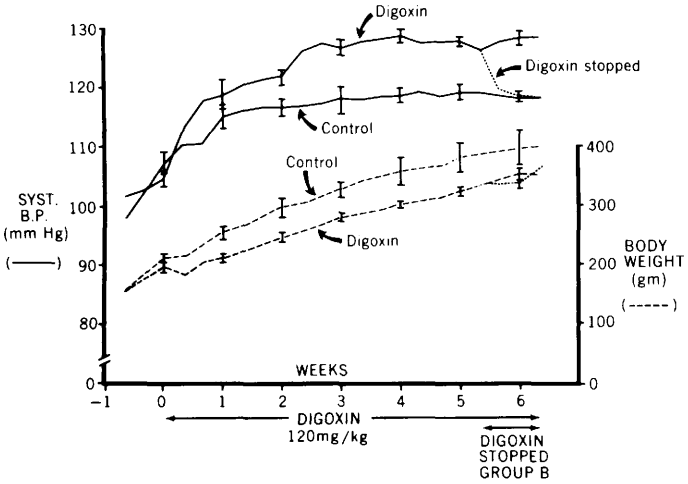

FIG. 1. Means ± SEM of body weights (dashed lines), and tail systolic blood pressures (unbroken lines). Curves identified as Digoxin represent pooled values in Group A and B rats (until digoxin was discontinued in Group B rats after 5 weeks (stippled lines)). Curves identified as Control represent values in Group C rats.

TABLE I. TISSUE WATER CONTENTS (% H_2O, M ± SEM)

	Group A	Group B	Group C	AoV[a]
Vena cava	67.96 ± 0.70[b] (N = 20)	64.23 ± 0.93[c] (N = 20)	65.78 ± 0.84[b,c] (N = 17)	<0.01
Aorta	62.82 ± 0.27[b] (N = 13)	62.46 ± 0.41[b] (N = 13)	62.52 ± 0.43[b] (N = 13)	>0.75
L. ventricle	76.98 ± 0.09[b] (N = 21)	76.81 ± 0.13[b] (N = 20)	76.82 ± 0.09[b] (N = 17)	>0.25
R. ventricle	76.94 ± 0.17[b] (N = 12)	76.89 ± 0.22[b] (N = 11)	76.77 ± 0.15[b] (N = 13)	>0.75

[a] Within each row, values sharing superscript letters are *not* significantly different (Newman–Keuls and/or analysis of variance).

pressures in Group A rats were increased compared to either Group B or C.

In Experiment 2, blood pressures and body weights taken three times per week during the first 2 weeks of digoxin administration revealed changes similar to those of Experiment 1. Again, systolic blood pressure rose by about 5% ($P < 0.01$; $N = 22$) within 3 days of the beginning of digoxin administration and remained elevated. Again, a defect (about 10%) in body weight appeared within 3 days and also persisted.

Tissue weights and water contents of rats from Experiments 1 and 2 not differing, were pooled for data analysis. Table I presents water contents of the walls of the thoracic portion of the inferior vena cava, the descending portion of the thoracic aorta and the ventricles in pooled rats of Experiments 1 and 2. Analysis of variance indicated significant differences in vena caval water content among the groups ($P < 0.01$). Newman–Keul comparisons indicated increases (3–5%) in vena caval water content in the rats on digoxin (Group A) compared to Group B, and differences of borderline ($P < 0.10$) significance between Groups A and C, but no significant differences between Groups B and C. Student's t test revealed highly significant increases in Group A rats ($P < 0.01$) when compared to pooled values in the two control groups (B and C), which, also by Student's t test, did not differ ($P > 0.25$). No differences were noted among the three groups in water content of the thoracic aorta, or left or right ventricles (Table I).

Table II presents pooled wet weight and wet weight per body weight of vena cava, aorta, and ventricles. No differences in vena caval or aortic weights were found among the groups. In contrast, however, analysis of variance revealed significant differences among the groups in left ventricular weight. Values in the two control groups (B and C) again did not significantly differ. On the other hand, there was good evidence that left ventricles from Group A rats treated with digoxin were significantly lighter (by 4–5% for weight/body wt) than those from the two control groups of rats. There were similar trends in the right ventricles (Table II) that were not statistically significant. However, right ventricular weight per left ventricular weight did not differ among the groups ($P > 0.75$), suggesting that there may have been accompanying attenuation of right ventricular growth.

Figure 2 presents the dose–response relation between total ^{86}Rb uptake and acute ouabain concentrations in thoracic aorta *in vitro*. Values of 0.4 μM (comparable to serum levels of 300 ng/ml) lie on the steep portion of the curve, so it is difficult to use these data to judge degree of inhibition we achieved in our rats *in vivo*. However, there is certainly no evidence that these concentrations of digoxin have any pump-stimulating effects in this system.

Finally, *in vitro* measurement of ^{86}Rb uptake by aorta freshly excised from Group A, B, and C rats with calculation of the ouabain-sensitive component of ^{86}Rb uptake (9) disclosed no significant differences: ouabain-sensitive uptake (M ± SEM),

TABLE II. TISSUE WEIGHTS AND WEIGHT/BODY WEIGHT (M ± SEM)

	Group A	Group B	Group C	AoV[a]
L. ventricle (mg)	653.2 ± 13.2[b] (N = 19)	697.7 ± 21.1[b,c] (N = 19)	722.8 ± 20.1[c] (N = 17)	<0.05
L. ventricle/body wt (mg/100 g)	173.3 ± 1.7 (N = 19)	183.3 ± 3.1[b] (N = 19)	182.3 ± 3.0[b] (N = 17)	<0.025
R. ventricle (mg)	191.3 ± 6.4[b] (N = 11)	216.0 ± 12.0[b] (N = 11)	207.8 ± 11.4[b] (N = 12)	>0.25
R. ventricle/body wt (mg/100 g)	49.6 ± 1.4[b] (N = 11)	53.2 ± 1.9[b] (N = 11)	52.1 ± 2.0[b] (N = 12)	>0.25
R. ventricle/L. ventricle ($\times 10^2$)	28.0 ± 0.8[b] (N = 11)	28.5 ± 1.1[b] (N = 11)	28.8 ± 1.2[b] (N = 12)	>0.75
Aorta/body wt (mg/100 g)	93.6 ± 5.0[b] (N = 16)	98.2 ± 3.3[b] (N = 11)	92.0 ± 3.0[b] (N = 17)	>0.5
Vena cava/body wt (mg/100 g)	2.68 ± 0.13[b] (N = 21)	2.56 ± 0.10[b] (N = 20)	2.74 ± 0.19[b] (N = 17)	>0.75

[a] Within each row, values sharing superscript letters are *not* significantly different (Newman–Keuls and/or analysis of variance).

Group A (N = 8), 3.21 ± 0.22 nmole/mg wet wt/10 min; Group B (N = 7), 3.33 ± 0.23; Group C (N = 4), 3.64 ± 0.40.

Discussion. Hendrickx and Casteels (11) have shown that the sodium pump of the sarcolemma of vascular smooth muscle may contribute up to 40% of the resting cell membrane potential. Intracellular sodium concentration, and, hence, cell volume (12), are also, in part, functions of the activity of this sodium pump. Intracellular sodium, by sodium–calcium interchange (13), and membrane potential, by voltage-dependent Ca^{2+} channels, have important roles in the regulation of intracellular concentrations of ionized calcium and, thereby, of the contractile state of the vascular smooth muscle cell and of the vascular resistance.

Based on experimental evidence (2, 3, 14–16), we have proposed (2, 3) that decreases in the activity of this ouabain-sensitive sodium pump in cardiovascular muscle may account, in part, for several manifestations of hypertension, including the increased pressure and resistance, the decreased venous compliance (17), the increased vascular responsiveness to certain vasoconstrictor agonists, and the vascular wall waterlogging (4, 5). If our hypothesis were true, supression of the pump in cardiovascular tissue of normal animals should result in similar manifestations. In this regard, there is prior evidence that digitalis increases peripheral vascular resistance (6, 7) and increases vascular responses to norepinephrine and nerve stimulation (18–20). We (1) recently presented evidence that chronic digoxin administration to dogs causes vascular wall waterlogging.

However, there has been no evidence that the chronic administration of digitalis

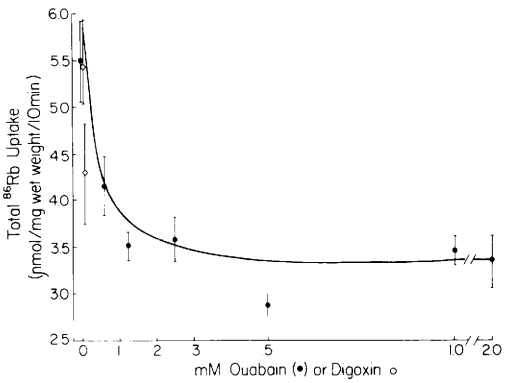

FIG. 2. Total Rb uptake (nmole/mg wet wt/10 min, mean ± SEM) by rat aortic segments *in vitro* vs ouabain concentration. Responses to two concentrations of digoxin are identified by open circles.

elevates blood pressure. Thus, in the present project we investigated the effects of chronic digitalis administration to rats. Blood pressure in conscious rats may be repeatedly measured with ease and small changes detected.

In these rats we achieved chronic serum digoxin concentrations of about 300 ng/ml (0.4 μM). In acute experiments we were unable to provide clear evidence that these levels produce an inhibition of the sodium pump in aortic smooth muscle. In the ouabain dose–response studies (Fig. 2), this was because the levels of interest lay on the steepest portion of the curve. In the studies of ^{86}Rb uptake, the fast rate of dissociation of digitalis from the smooth muscle membrane in this species undoubtedly played a role in obscuring any inhibition present, as tested *in vitro*. The result of these acute exposures to digitalis by no means preclude the possibility that we produced chronic inhibition of the vascular sodium pump by feeding digoxin to our rats. Vena caval tissue was not tested because of the far lesser weight of vena cava and difficulty of dissection.

Measurement of tail systolic blood pressure three times per week revealed highly significant increases in arterial pressure associated with digoxin treatment. These increases occurred within three days and continued at levels about 6% higher than normal during the entire 5- to 7-week period of digoxin administration. When digoxin was discontinued in some of these rats (Group B), their arterial pressures returned to normal levels within three days. We observed these blood pressure elevations in two separate experiments involving a total of 41 rats. Our experiments were conducted in young growing animals, which have been found to be more sensitive than older rats to other hypertensinogenic stimuli. Similar measurements in older rats would be of great interest.

We interpret these increases in arterial pressure in our digoxin-treated rats as compatible with our hypothesis that pump depression in cardiovascular muscle may account in part for the elevated blood pressure in certain forms of hypertension. It is noteworthy that there is recent evidence for elevated levels of a digitalis-like humoral substance, "endoxin" (21), in certain forms of experimental hypertension (22). Acute *in vitro* exposure to endoxin may increase microvascular responsiveness to norepinephrine (23).

The present study was not designed to characterize the hemodynamic state underlying these digoxin-induced rises in arterial pressure in our rats. Although it is well documented that digitalis increases peripheral vascular resistance at least acutely, as noted above, we cannot exclude a role for increased cardiac output, or even blood viscosity. Furthermore, even if elevated peripheral resistance accounted for the rise in pressure, we cannot be certain that the vascular smooth muscle contraction was directly induced by digitalis, rather than, for example, the result of digitalis-evoked neurogenic vascular stimulation.

In the present study we also observed vascular wall waterlogging in the digoxin-treated animals, as in our previous investigation in dogs (1). This waterlogging was quantitatively similar to that observed in certain forms of hypertension (4, 5). Because this 3–5% increase in wall water content occurred in veins in the present study, it is unlikely to be the direct result of elevated intravascular pressure. As in the digoxin-treated dogs (1), we suggest that the waterlogging represents increases in cell water content produced by chronic inhibition of the cell membrane sodium pump (12). In contrast to dogs, we found no evidence for increases in wall water content of the arteries. However, in the rats we studied aorta, whereas in the dogs we found waterlogging of the mesenteric arteries, so the results are not strictly comparable. In rats, the association of waterlogging with elevated arterial pressure is, by no means, evidence for causal relationship; the role of vascular wall waterlogging in the production of the abnormal hemodynamic state in hypertension remains unclear.

An additional finding of the present investigation was the growth defect induced by digoxin in these young rats. Body weight dropped by 13% shortly after digoxin was begun. Thereafter, the rats remained lighter, but rate of weight increase was

normal. We were unable to determine whether this defect in body weight was reversible. Accompanying the defect in body weight in the digoxin-treated rats was evidence for a defect in cardiac growth. Left ventricular weight was reduced although the afterload was elevated. This defect was present even when heart weight was expressed on a per body weight basis; thus, heart growth was decreased even more than body growth was decreased. This defect in cardiac growth may have been reversible, because heart weights were within normal limits in the Group B rats within 1–2 weeks after digoxin was discontinued. In contrast to the hearts, we observed no evidence for similar defects in the growth of major arteries or veins in our digoxin-treated rats.

We are unaware of any previous studies observing defects in cardiac growth associated with chronic digitalis administration to normal animals. Cutilletta *et al.* (24) administered parenteral digitoxin to normal adult rats and did not find significant decreases in left ventricular weight; however, there were trends in that direction and digitalis was administered to only six normal rats. Williams and Braunwald (25) treated larger numbers of normal rats with parenteral digitoxin and also noted no change in ventricular weight per body weight. However, duration of treatment of their rats was from 3 to 6 weeks less than that in the present study. Furthermore, our rats received digitalis every day, whereas in these two previous studies the daily injections were omitted 1 day each week.

As a related observation, it has been previously reported that digitalis administration attenuates (25, 26) or prevents (27) pressure-induced left ventricular hypertrophy, although there is controversy (24). This reduced hypertrophy has not been explained, despite suggestions that digitalis may enable the normal myocardial mass to meet the increased pressure work, or that digitalis interferes with myocardial growth by impeding sodium pump-linked amino acid transport across the cell membrane (27–29). These mechanisms may also explain the defect in myocardial growth we observed. However, decreases in cardiac preload, changes in cardiac rate, decreased sympathetic stimulation, or other factors depressing intramyocardial metabolism, and hence growth, may also have contributed.

Dr. David D. Ku participated in some of these experiments. Dr. H. B. Otwell measured digitalis dose–response relationships in rat aorta. Drs. S. Oparil and J. B. Smith reviewed the manuscript and supplied helpful comments and suggestions.

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Received January 6, 1980. P.S.E.B.M. 1981, Vol. 167.