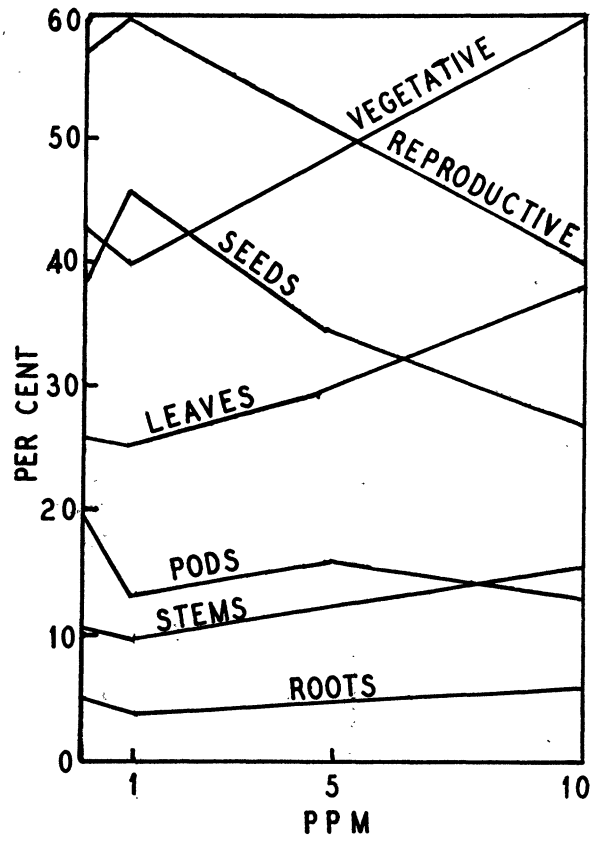

FIG. 2. Effect of 2,4-D on the dry weight of the various organs of bean plants and of the total vegetative and reproductive organs, expressed as percentage of the total dry weight.

under surfaces of some of the most deformed leaves. These were similar to those reported by Felber (*3*) but had developed during March and April, while Felber found such proliferations on the lower epidermis regularly only from June to October.

The 2,4-D appeared to promote, rather than to delay or inhibit, leaf abscission. Leaf abscission began on the controls and plants in all treatments on March 30 but was completed a weak earlier in the plants sprayed with 5 or 10 ppm than on the controls or plants sprayed with 1 ppm. The abscission of leaves from all plants reached a peak between April 11 and 14, and the rate during this peak period was higher for all treated plants than for the controls.

All controls and plants which received 1 ppm, and two plants which received 5 ppm began blooming on March 8, but none of the plants sprayed with 10 ppm bloomed until a week later. The number of macroscopic flower buds and flowers on March 10 is given in Fig. 1, and the data on pods in this figure provide additional evidence of the delaying effects of the 5 and 10 ppm concentrations on reproductive development. That this repression of reproductive development was not due to a general repression of growth is indicated by the increased vegetative development of the treated plants (Figs. 1 and 2). Some of the flowers on the plants sprayed with 10 ppm were deformed, having greatly reduced petals, elongated and exserted styles, and ruptured ovaries. The pods of the plants sprayed with 5 and 10 ppm were unusually dark green, and even those on plants sprayed with 1 ppm were darker green than the controls.

None of the treatments had a significant effect on total dry weight of the plants, but there were significant differences in the dry weights of the various organs (Fig. 2). The 5- and 10-ppm concentrations definitely produced a decrease in the dry weight of the reproductive organs and a concurrent increase in the dry weight of the vegetative organs. The 1-ppm concentration, however, produced a significant effect which was the reverse of this, due principally to the unusually high seed weight in this treatment. This was not due to a greater weight per seed, nor to a larger total number of seeds, but to a larger number of fully mature seeds (Fig. 1). In general, the weight per seed decreased with an increase in concentration. (The mean weight of the mature seeds of the controls was 0.328 gm, and mean weights of the seeds of the plants treated with 1, 5, and 10 ppm were 0.286, 0.337, and 0.259 gm, $\pm$ 0.020). There is no evident explanation for the high weight per seed in the 5-ppm treatment. The increase in the total dry weight of the leaves with concentration of 2,4-D was due to the increase in the number of leaves, rather than to an increase in weight per leaflet, which actually decreased with higher concentrations. It seems possible that the delaying and inhibiting effects of 2,4-D on reproductive development reported here and by other investigators are the same type of phenomenon as the inhibition or delay action of other growth substances, reported by Dostal and Hosek (*2*), Obsil (*5*), Galston (*4*), and others.

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The Effect of Castration and of Testosterone upon the Respiration of Rat Brain

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In an earlier paper (*3*) it was reported that various steroids are capable of inhibiting the oxygen uptake of rat brain homogenates. It was found that testosterone inhibits the oxidation of glucose at some point preceding participation of the cytochromes in "the main line of

biological oxidation'' (*2*). In an attempt to determine whether or not testosterone is concerned with normal oxidative mechanisms, we have compared the respiration of tissues of castrated rats with the respiration of tissues of normal and testosterone-treated castrated rats.

Male rats of the Long-Evans and Slonaker-Wistar strains were used. There were no significant differences between data derived from the two strains and therefore the results were pooled. Tissue respiration studies were performed upon three groups of animals: a group of normal males, a second group castrated at the age of 30 days, and a third group castrated at 30 days and afterward treated subcutaneously with 1 mg of testosterone propionate[1] per day. The rats were decapitated at 60 days of age and the QO_2 of brain was determined by the direct method of Warburg. Cell suspensions of cerebral hemispheres were prepared by the method of Potter and Elvehjem (*6*) in ice-cold modified Krebs' Ringer-phosphate buffer containing 0.2% glucose as substrate. One ml of a 10% homogenate was placed in a Warburg flask containing sufficient buffer and material in the sidearm to make a final volume of 2 ml. The vessels were placed in a bath at 37.2° C within 10 min after decapitation. The gas phase was oxygen. Each experiment lasted 3 hr with readings taken at 15-min intervals. When testosterone was added *in vitro*, 2 mgm of a fine suspension was added after a 90-min control period. QO_2 is reported as μl of oxygen per mgm of dry brain per hr. Wet/dry ratios were determined by the method of Crismon and Field (*1*).

Experimental data are summarized in Table 1. It will be observed that the brain of the castrated rat has an oxygen uptake 32% higher than that of the normal rat (p value < 0.01). The brain of the castrated rat treated with testosterone respires at a rate only slightly higher than normal ($p < 0.01$). The addition of testosterone *in vitro* further suppresses the oxygen uptake of all groups; however, the inhibition is far less in the castrated than in either the treated castrated or the normal group. It will further be observed that the QO_2 of the brain of the untreated castrated rat to which testosterone has been added *in vitro* (5.3) is essentially the same as that of the testosterone-treated castrated rat brain (5.4) at a comparable time. The rate of oxygen uptake by the brain of the treated castrated rat can be decreased to a still lower level (2.6) by the further addition of testosterone *in vitro*. The variations in QO_2 cannot be explained by difference in water content, since the wet/dry ratios of all groups are essentially the same.

TABLE 1

	Number of animals	Body weight gm ± σm*	Weight brain mgm ± σm	Weight brain per 100 gm body weight	Mean QO_2 first 90 min μl ± σm	Final QO_2 180 min (no testosterone) μl ± σm	Final QO_2 180 min (testosterone added at 90 min) μl ± σm	Inhibition of QO_2 produced by testosterone	Wet/dry ratio
Normal	12	182 ± 8.1	1206 ± 29	663	6.5 ± 0.1 (35)†	4.3 ± 0.1 (25)	1.5 ± 0.1 (10)	65%	4.9
Castrate untreated	16	161 ± 4.1	1152 ± 21	716	8.6 ± 0.1 (72)	6.8 ± 0.1 (30)	5.3 ± 0.1 (42)	22%	4.7
Castrate treated	13	166 ± 8.3	1094 ± 26	659	6.9 ± 0.1 (80)	5.4 ± 0.1 (28)	2.6 ± 0.1 (52)	52%	4.7

* σm—Standard deviation of the mean. †—Number of vessels in parentheses.

Inasmuch as the potential enzymatic activity of certain tissues exceeds the actual activity found *in vivo*, the concept of the metabolic ''brake'' has been postulated (*4*, *5*). It appears that testosterone exerts such a ''braking'' action upon the oxidation of glucose by the rat brain. This is borne out by the high QO_2 of the castrated rat brain and the prevention of the rise of QO_2 by testosterone treatment *in vivo*. The further addition of testosterone *in vitro* is capable of lowering the QO_2 to subnormal levels in normal and testosterone-treated castrates. This is not the case with untreated castrates. In other words, the brain of the castrated rat is less sensitive to the oxidation-inhibiting effect of testosterone *in vitro* than is the brain of the normal rat or of the castrated rat treated with testosterone. A possible interpretation is that the intracellular concentration of the enzyme system by which testosterone exerts its inhibiting effect upon the oxygen uptake of brain is decreased after castration. This decrease can be prevented for the most part by the administration of testosterone.

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