of the notation used in this report was:

$$w_0^{1/3} - w_\tau^{1/3} = \frac{50\tau}{(\sigma/2.303)^{1.05}\,M} = \alpha\tau \qquad \text{(Eq. 19)}$$

where M is the geometric mean diameter of the distribution and the sense of α is obvious. This equation, rearranged to give:

$$w_\tau/w_0 = [1 - (\alpha/w_0^{1/3})\tau]^3 \qquad \text{(Eq. 20)}$$

was tested for its fidelity. This was done by calculating α and then finding a value for $w_0^{1/3}$ by plugging in a point τ^*, w_τ^*/w_0 from the exactly calculated profile. Then the entire dissolution pattern was calculated from Eq. 20 and compared with the exactly calculated values. These comparisons are shown in Table II for several different powder distributions. The point τ^*, w_τ^*/w_0 is indicated in Table II. For standard deviations that are small or of moderate size, the approximation of Carstensen and Musa as rearranged in Eq. 20 gives excellent values when compared with the precise dissolution curve even for values $\tau > \tau_c$. For large σ, the approximation is not quite as good. In those distributions where dissolution is essentially complete before $\tau = \tau_c$, Eq. 20 can be employed without a knowledge of the exact profile. The initial condition can be set by $(w_\tau/w_0) = 0$ at $\tau = \tau_c$. This works well for the distribution where only 0.1% of the initial weight remains at τ. It does not work well for the distribution where 2.56% of the weight remains at τ_c (Table II).

The growing importance of dissolution rate technology in pharmacy, coupled with the fact that many powders follow the log-normal distribution law, should lend importance to the exact equation derived here. In practical terms, however, the use of the exact equation for the dissolution of log-normal powders requires a knowledge of population parameters, solubility, and dissolution rate constant, none of which may be readily available. The population parameters found may depend on the method of measurement. The solubility of the smallest particles should be greater than that of the largest particles. Few powders are composed of spherical particles that dissolve isotropically. And, finally, it would undoubtedly be difficult to design a dissolution rate experiment in which the agitation and, therefore, the diffusion barrier were the same for the entire surface of each particle and for every particle.

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Chronic Isoproterenol Treatment of Mice: Effects on Catecholamines and Rectal Temperature

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Abstract □ The chronic administration of isoproterenol (5 mg./kg. twice a day) to male and female mice resulted in increased wet weights and tissue weight–body weight ratios of the submaxillary and parotid glands. For the heart the ratio was elevated in male but not in female mice. The first dose of isoproterenol produced a decrease in the rectal temperature. Continuation of the treatment led to hyperthermia, which became maximum after treatment for longer than 10 days (20 doses). In these animals the predose rectal temperatures were lower than the pretreatment control values and the temperatures of control animals. A smaller, single dose (2.5 mg./kg.) did not alter the rectal temperature. The norepinephrine concentrations of the parotid and submaxillary glands were reduced in male and female mice, but the total norepinephrine content of these glands was decreased only in male mice. The cardiac norepinephrine levels were not affected. Chronically treated animals were less active than controls for about 90 min. after dosing and showed rarification of fur and hair loss. Isoproterenol treatment of dams before, during, and after pregnancy did not alter the body weight, gross appearance, and wet weight of organs and tissues of pups examined on the 3rd postnatal day.

Keyphrases □ Isoproterenol—effect on catecholamines and rectal temperature after chronic administration, mice □ Catecholamine concentration—effect of chronic isoproterenol administration, mice

A number of investigators have reported physiological and pathological changes in various species following acute and chronic isoproterenol treatment (1–14). Depending on the species and duration of treatment, the reported effects have included hypertrophy and hyperplasia of the submaxillary and parotid glands, infarct-like lesions and fatty degeneration of the heart, growth stunting, premature opening of the eyes, sparse hair growth, prostration, lethargy, and changes in rectal temperature, norepinephrine levels, and wet weights of several peripheral tissues.

To gain a better understanding of the mode of action of isoproterenol, we investigated several effects of chronic treatment in mice, a species in which this drug has not been studied in detail. The factors studied included the wet weights and norepinephrine levels of several tissues and organs and the effects of acute and chronic administration on the rectal temperature.

METHODS

Animals—In one study, 16 adult male Swiss Webster mice were injected subcutaneously twice a day, except Sundays, with 5 mg./kg. isoproterenol hydrochloride and another 16 mice were injected with 0.85% sodium chloride solution for 15, 23, and 31 days. The animals were sacrificed by exsanguination under sodium pentobarbital anesthesia. Whole brain (minus the cerebellum) or brain-

Table I—Ratios of the Tissue Weight to the Body Weight (mg./g.) of the Heart, Parotid Glands, and Submaxillary Glands in Male and Female Mice, Treated Chronically with Isoproterenol Hydrochloride (ISO)

Treatment and Body Weight, g. ± *SE*	Sex	Number of Mice	Ratio, Tissue Weight/Body Weight, mg./g. ± *SE*		
			Heart	Parotid Glands	Submaxillary Glands
Control, 31.1 ± 0.9	Male	9	3.9 ± 0.1	3.7 ± 0.2	5.2 ± 0.2
ISO, 29.4 ± 0.5	Male	9	5.4 ± 0.1[a]	12.0 ± 0.4[a]	11.0 ± 0.5[a]
Control, 32.6 ± 2.5	Female	7	4.6 ± 0.2	3.3 ± 0.5	5.1 ± 0.5
ISO, 43.6 ± 1.0	Female	8	4.8 ± 0.1	14.5 ± 1.6[a]	8.9 ± 0.5[a]

[a] $p < 0.001$ treated to control of the same sex, *t* test in nonpaired experiments (29).

stem[1], heart, submaxillary glands, parotid glands, spleen, kidneys, small intestine, and adrenal glands were dissected in the cold and weighed on a Mettler balance or Cahn electrobalance. The tissues were either immediately analyzed for catecholamines or frozen for subsequent measurement, always within 3 days after dissection.

In the second study, 25 female Swiss Webster mice were injected subcutaneously twice a day with 0.85% sodium chloride solution (eight controls) or with 5 mg./kg. isoproterenol hydrochloride for 65 days (10 mice) and 107 days (two mice) before, during, and after pregnancy. Five isoproterenol-treated mice died during the treatment. The mice were sacrificed, and the same tissues were dissected and prepared as already described.

Neonatal mice were sacrificed 3 days after birth, and the peripheral organs and tissues, including sympathetic ganglia, were examined grossly and weighed on a Mettler balance or Cahn electrobalance.

Tissue Weights and Ratios—For the statistical analysis of the effect of chronic isoproterenol treatment on the tissue weights, the ratios of the tissue weights to the body weights (milligrams per gram) were used. There were no differences in the body weights within and between the groups of treated and control male mice. The body weights of the female control mice were lower than the body weights of the isoproterenol-treated female mice before treatment and remained so throughout the experiment.

Catecholamine Analysis—Norepinephrine and epinephrine were extracted with 1-butanol as previously described (15) and measured in a spectrophotofluorometer[2] by a modification (16, 17) of the ferricyanide oxidation method of von Euler and Floding (18).

Rectal Temperature—The rectal temperatures of female mice were measured with a telethermometer[3] every 30 min. for up to 150 min. (room temperature 22–24°) before and after the administration of the first and third doses of isoproterenol or 0.85% sodium chloride solution. During the chronic treatment, the rectal temperatures were determined periodically. Rectal temperatures were measured in another group of female mice following a single lower dose (2.5 mg./kg.) of isoproterenol hydrochloride.

Other Observations—During the entire experimental period the gross appearance of chronically treated male and female mice was observed. These observations included the appearance of the fur and the occurrence and severity of lethargy and salivation after dosing. The results of the histopathological study of tissues from isoproterenol-treated and control mice will be reported elsewhere.

Within the groups of female and male mice, no variation in the intensity of the effects on the tissue weights, the ratios, and the tissue catecholamines was noted that could be attributed to the different durations of the chronic treatment. For this reason, these data were pooled (65 and 107 days for female mice and 15, 23, and 31 days for male mice).

RESULTS

Tissue Weights—The wet weights of the hearts, submaxillary glands, and parotid glands were larger in the treated mice of both sexes than in the control mice. In treated male mice the ratio of the heart weights to the body weights was significantly increased, while this ratio was not altered in the female mice. The ratio was larger in female control mice when compared to male control mice ($p < 0.01$); the opposite was noted in the isoproterenol-treated male and female mice ($p < 0.001$). The ratios of the parotid and submaxillary glands from isoproterenol-treated mice of both sexes were significantly increased over control values (Table I). The ratios of parotid glands from male and female treated mice were not statistically different, but those of the submaxillary glands were larger in male than in female treated mice ($p < 0.01$). In control male and female mice the gland to body weight ratios were similar. Spleen, intestine, kidneys, adrenal glands, superior cervical ganglion, brain, and brainstem showed no alteration in the tissue weight to body weight ratios as a result of chronic isoproterenol treatment. Gross appearance and the wet weights of tissues of neonatal animals from isoproterenol-treated female mice were not different from those of neonatal controls.

Rectal Temperature—A decrease in the rectal temperature of female mice occurred 30, 60, 90, 120, and 150 min. after the administration of a single dose of 5 mg./kg. isoproterenol when the experimental temperatures were compared to the predrug control values and control mice (Table II). After three doses the hypothermic response was no longer demonstrable, and continuation of the isoproterenol treatment led to hyperthermia. The rectal temperature increased significantly within 30 min. after dosing, remained elevated for approximately 1 hr., and then began to return to the control value. The maximum increase in the rectal temperature was not reached at 7 (Table II) or 10 days but was observed after 29 days of treatment. The final temperature measurements were taken 24 hr. before sacrifice after 65 and 107 days of treatment. The mean increase amounted to 2.2° with a range from 1.8 to 3.2°. In half of the mice, peak temperatures were reached at 30 min.; in the other half they were reached at 60 min. after the administration of the dose. During the chronic treatment the rectal temperatures recorded before the administration of a daily dose of isoproterenol were lower than the temperatures of these mice before the chronic treatment was started, during the earlier periods of the treatment, and in control mice; these differences were statistically significant. Treatment of female mice with a single dose of 2.5 mg./kg. isoproterenol did not affect the rectal temperature during the observation period (Table II).

Catecholamines—The norepinephrine concentration of the parotid glands was significantly decreased in treated mice of both sexes when compared to their respective controls. The norepinephrine content of these glands was also decreased in chronically treated male mice but not in the treated female mice. In female but not in male mice, isoproterenol treatment reduced the epinephrine concentration of parotid glands by 28%. A fourfold increase in the total epinephrine content (nanograms per glands) occurred in the parotid glands of both male and female treated mice.

Chronic isoproterenol treatment reduced the norepinephrine concentration to 37% and the content to 80% of control values in the submaxillary glands of male mice. Female treated mice also showed a decreased norepinephrine concentration (to 69% of control value), but the norepinephrine content was increased to 163%. The norepinephrine concentration and content of the submaxillary glands from male control mice were 2.5-fold greater than those from female control mice. Lower norepinephrine concentration and content of parotid glands were also noted in female control than in male control mice (Table III). Similar differences were obtained in a subsequent study which was undertaken to verify these findings. The epinephrine concentration and content of the submaxillary glands from male mice were greater regardless of the treatment when compared with the respective glands from

[1] Brainstem is designated as the medulla, the pons, the midbrain, and the diencephalon.
[2] Aminco-Bowman.
[3] Yellow Springs Instrument Co., probe No. 402.

Table II—Rectal Temperatures of Female Mice Treated Subcutaneously with 5 mg./kg. Isoproterenol Hydrochloride (ISO)

Treatment	Number of Mice	Rectal Temperature ± *SE* before and Every 30 min. after Treatment					
		0	30	60	90	120	150
Controls	13	38.1 ± 0.1°	38.1 ± 0.1°	37.9 ± 0.1°	37.8 ± 0.1°	37.7 ± 0.1°[a]	37.7 ± 0.1°[a]
First dose ISO	14	38.1 ± 0.1°	37.2 ± 0.2°[4d]	37.4 ± 0.2°[1c]	37.2 ± 0.2°[3d]	37.2 ± 0.1°[3d]	37.3 ± 0.1°[1d]
First dose ISO, 2.5 mg./kg.	11	38.1 ± 0.2°	37.9 ± 0.2°	37.7 ± 0.3°	37.5 ± 0.3°	37.4 ± 0.3°	37.3 ± 0.3°[a]
Third dose ISO	14	38.1 ± 0.2°	37.8 ± 0.2°	37.7 ± 0.2°	37.6 ± 0.1°	37.5 ± 0.2°[a]	37.3 ± 0.2°[b]
Controls	11	37.6 ± 0.2°	37.4 ± 0.2°	37.2 ± 0.2°	37.2 ± 0.2°	—	—
7 days ISO	14	37.7 ± 0.1°	39.1 ± 0.2°[4d]	39.0 ± 0.2°[4d]	38.6 ± 0.2°[4d]	—	—
65–107 days ISO	12	36.8 ± 0.1°[3]	39.0 ± 0.3°[4d]	38.6 ± 0.2°[4d]	37.9 ± 0.2°[1d]	—	—

[1] $p < 0.05$, [2] $p < 0.02$, [3] $p < 0.01$, [4] $p < 0.001$ } *t* test in nonpaired experiments (29), treated to control.

[a] $p < 0.05$, [b] $p < 0.02$, [c] $p < 0.01$, [d] $p < 0.001$ } *t* test in nonpaired experiments (29), zero-time temperature (predrug) to experimental temperature (postdrug).

Table III—Norepinephrine and Epinephrine Concentration and Content of Tissues of Male and Female Mice Treated Chronically with Isoproterenol Hydrochloride (ISO)

Tissue	Number of Mice	Treatment	Sex	Norepinephrine		Epinephrine	
				ng./g. ± *SE*	ng./Tissue ± *SE*	ng./g. ± *SE*	ng./Tissue ± *SE*
Submaxillary glands	9	Control	Male	4320 ± 330[4d]	686 ± 36[1d]	192 ± 28[b]	31 ± 5[4a]
	9	ISO		1610 ± 165[4c]	527 ± 63[1]	226 ± 29[c]	75 ± 9[4b]
	7	Control	Female	1690 ± 95[3d]	270 ± 19[4d]	105 ± 17[b]	17 ± 3[a]
	8	ISO		1170 ± 99[3c]	442 ± 29[4]	99 ± 23[c]	38 ± 10[b]
Parotid glands	9	Control	Male	1270 ± 120[4c]	141 ± 7[4c]	120 ± 12	14 ± 1[4]
	9	ISO		238 ± 21[4]	82 ± 5[4]	142 ± 25	50 ± 8[4]
	7	Control	Female	827 ± 73[4c]	86 ± 15[c]	120 ± 10[3]	12 ± 1[4]
	8	ISO		173 ± 44[4]	91 ± 17	87 ± 5[3]	54 ± 8[4]
Heart	9	Control	Male	545 ± 59	65 ± 6	65 ± 12[3]	8 ± 2
	9	ISO		467 ± 37	74 ± 6[c]	25 ± 5[3]	4 ± 1
	7	Control	Female	492 ± 37	76 ± 12[3]	72 ± 17	10 ± 2
	8	ISO		556 ± 49	115 ± 10[2c]	48 ± 16	10 ± 3

[1] $p < 0.05$, [2] $p < 0.02$, [3] $p < 0.01$, [4] $p < 0.001$ } *t* test in nonpaired experiments (29), control to treated of the same sex.

[a] $p < 0.05$, [b] $p < 0.02$, [c] $p < 0.01$, [d] $p < 0.001$ } *t* test in nonpaired experiments (29), control male to control female or treated male to treated female.

female mice. Chronic isoproterenol treatment doubled the total epinephrine content (nanograms per glands) of the submaxillary glands of both sexes.

The cardiac norepinephrine concentration was not significantly different in control and isoproterenol-treated mice of the same sex nor in control animals of opposite sexes. However, treated female mice showed a significantly higher norepinephrine content of the heart than the female controls and the treated males (Table III). In male mice, chronic isoproterenol treatment reduced the cardiac epinephrine concentration.

The brainstem norepinephrine and epinephrine of male mice were not altered by chronic isoproterenol treatment. Chronic treatment of female mice, in which the catecholamines of the whole brain were measured, resulted in an increase in norepinephrine concentration (127% of control value; $p < 0.05$). No change in the brainstem epinephrine concentration was noted. The adrenal norepinephrine concentration of chronically treated female mice showed a mean increase to 164%; this was statistically nonsignificant. The adrenal epinephrine concentration was not altered. The adrenal catecholamines of control and treated male mice were not measured. Concentrations of norepinephrine and epinephrine in spleen, intestines, and kidneys were similar to those of control animals.

Other Observations—After the first few doses, the mice of both sexes were less active than the control animals for approximately 60–90 min. following administration of isoproterenol. There was little or no salivation. Continuation of the treatment accentuated the depression and salivation became marked. Rarification of fur and hair loss, resulting in bald areas, were also noted. The number of pups per litter produced by the chronically treated dams was similar to the litter sizes of controls. The body weights, the gross appearance, and the wet weights of the organs and tissues of pups from isoproterenol-treated dams were not different from those of control animals when examined on the 3rd postnatal day.

DISCUSSION

The histopathological and biochemical effects of isoproterenol on the peripheral tissues of mice have not been explored to the same extent as in the rat. The weight increases of the submaxillary and parotid glands of mice were similar to those reported in rats (3, 5, 13, 14). According to Brown-Grant (11), large doses (30 and 40 mg./100 g.) in mice produced hypertrophy but no hyperplasia of submaxillary and parotid glands. Baserga (12) found a 10-fold increase in DNA synthesis and stimulation in cell proliferation in submaxillary glands of mice after treatment. It is not known whether isoproterenol treatment produces histopathological lesions in the mouse heart comparable to those observed in the rat. In the current study, an increase in the wet weight of the heart was demonstrated in male mice only. This increase, expressed as the ratio of the tissue weight to the body weight, amounted to 138% of the control value ($p < 0.001$). In rats treated chronically with 10–15 mg./kg. isoproterenol twice a day, the ratio increased in male and female animals (145.2 and 147.5%, respectively, of control values) (13).

A similar pattern of hypothermic and hyperthermic responses following single and repeated doses of isoproterenol, as seen in the current study with mice, has also been noted in rats (13, 19). In both species, chronic treatment for longer than 10 days was required before a maximum hyperthermic response (increase of 2–3°) was reached. In the group of male mice, no temperature measurements were made. However, isoproterenol treatment affected the rectal temperature similarly in male and female rats (13).

A smaller, single dose, 2.5 mg./kg., did not produce hypothermia (Table II). From the data obtained in the current study with mice and in another study with rats (19), one may conclude that the effect of a single dose of isoproterenol on the rectal temperature is dose related. As shown by Brittain and Handley (20), the intracerebral injection of isoproterenol resulted in an increase in esophageal temperature with small doses, 5–20 mcg./mouse, and a

decrease with a larger dose, 200 mcg./mouse. These authors suggested that the adrenergic α-receptor is involved in the hypothermic response and that the adrenergic β-receptor is involved in the hyperthermic response. Feldberg and Myers (21) postulated that hypothermia is the result of centrally released norepinephrine. Evidence for and against this proposed mechanism is available. For instance, reduction of central norepinephrine antagonized the 6-hydroxydopamine-induced hypothermia in the cold (22) but not at room temperature (23).

In the rat the cardiac norepinephrine concentration and content were significantly reduced after acute and short-term isoproterenol treatment (14, 24, 25). Mueller and Axelrod (25) presented evidence indicating that the reduction of the cardiac norepinephrine may be due to a storage defect. The nerve endings of the heart responded with a normal uptake but showed a decreased capacity to retain labeled norepinephrine. These authors linked the storage defect to an imbalance of the cardiac electrolytes, particularly sodium. An increase in sympathetic activity is believed to contribute to the decreased cardiac norepinephrine level.

After chronic isoproterenol treatment of rats only the norepinephrine concentration—not the total content of the heart—was significantly decreased (13). The fact that the cardiac norepinephrine concentrations in mice were not affected by chronic isoproterenol treatment, even though there was an increase in the heart weight of male mice, might indicate differences in species, sex, and/or long-term treatment (>1 month) to the effects of the drug that require further study. The statistically significant increase in the cardiac norepinephrine content in female treated mice (Table III) complicated the interpretation of the data even more in regard to the state of endogenous norepinephrine after chronic and short-term isoproterenol treatment.

It is not known whether a similar mechanism, as postulated by Mueller and Axelrod (25), may be responsible for the norepinephrine reduction found in the submaxillary and parotid glands after isoproterenol treatment. With certain dose regimens in rats, the extent of the reduction of the norepinephrine concentrations in these glands was similar to that in the heart, but the effect on the norepinephrine content of the glands by acute and short-term isoproterenol treatment was statistically nonsignificant (14). However, in chronically treated male mice the norepinephrine content of the submaxillary and parotid glands was reduced ($p < 0.05$ and 0.001, respectively), while in treated female mice the norepinephrine content of the submaxillary glands was elevated and no change was noted in parotid glands.

The lethargy observed with mice in the present studies and with rats (13) following isoproterenol treatment may have been described previously. Bertaccini *et al.* (2) reported prostration in rats after the administration of 400 mg./kg. of isoproterenol for 12 days. Fukuda (26) used the same term to describe the effect produced in rats by 5 mg./kg. administered once a day for 14 days. In Fukuda's study the prostration apparently occurred with the first dose and did not become more marked with subsequent dosing. In the present studies the lethargy following the first dose of isoproterenol was not as severe as that seen after repeated doses, particularly with long-term chronic treatment, and was not as pronounced in mice as in rats. However, the mice received only 5 mg./kg. while the rats received 15 mg./kg. (13). The rarification of the fur was less marked in the chronically treated mice than in the rats (13, 19). This also might have been due to the smaller dose administered chronically to the mice.

Five of the 17 female mice died in the course of the chronic treatment. Compared to the acute intraperitoneal LD_{50} in female Swiss Webster mice (250 mg./kg.; 95% *CL* 221–283; mice were observed for 1 week; death occurred within 18 hr.[4]), the dose of 5 mg./kg. used in these studies is very low. No deaths occurred in the group of 16 male mice treated with isoproterenol. According to Barka (27), male rats were more sensitive than female animals to the lethal effect of a three-dose course of intraperitoneally injected isoproterenol.

According to Geber (9), single doses of isoproterenol (0.003–34.8 mg./kg.) administered subcutaneously to pregnant hamsters on the 8th day of gestation produced a variety of anomalies and the percentages of dead, resorbed, and runt fetuses were increased over those in control litters. Vogin *et al.* (28) exposed pregnant rats and rabbits to isoproterenol aerosol to provide daily dosage levels of 150–450 mcg./kg. There was no evidence of teratologic effects due to the drug or the methodology. The present study in mice supports the observations reported by Vogin *et al.*, since no gross abnormalities were found in neonatal mice and their organs and tissues. However, histopathological lesions cannot be ruled out. The tissues of the neonatal animals were not examined histologically.

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