Effect of Tolbutamide on Plasma Free Fatty Acids and Blood Sugar in Birds[1] (35618)

FRANCISCO GRANDE

Jay Phillips Research Laboratory, Mount Sinai Hospital, and Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Minnesota 55404

In addition to their hypoglycemic effect, tolbutamide (1-butyl-3-(*p*-tolylsulfonyl)urea) and other sulfonylureas cause a sharp decrease of plasma free fatty acid (FFA) concentration in man and dog (1–6), which has been attributed to the release of insulin induced by these compounds (1, 2, 7). Other observations, however, suggest a direct antilipolytic effect of these drugs (8, 9). Tolbutamide has been shown to produce a decrease of blood sugar (BS) in ducks, geese, domestic fowls, and 1-day-old turkeys (10–13), but there is no information regarding its effect on the plasma FFA levels of birds. Administration of insulin does not produce a decrease of plasma FFA in several avian species (14–18), and tolbutamide has been reported to suppress the release of glucagon in the duck (19). For these reasons, and because of the marked adipokinetic effect of glucagon in birds (20, 21), it is of interest to investigate the effect of tolbutamide on the plasma FFA concentration of these animals. Accordingly, a study was made of the effect of this drug on the plasma FFA of intact birds and of birds whose plasma FFA levels had been elevated by the continuous intravenous infusion of glucagon (20, 21).

Methods. Adult, male, and nonlaying female, domestic geese (Embden and Toulouse) and ducks (Peking and Muscovy), and horned owls, were used in these experiments. They were kept and fed as previously described (17, 20, 21). Experiments were performed after 16–18 hr of fasting. Tolbutamide sodium[2] was injected intravenously. Crystalline glucagon[3] was dissolved just before the experiments in glycine buffer (0.02 *M*, pH 9.5, in saline) and infused with a continuous syringe pump through a catheter inserted into a wing vein (21).

Plasma FFA were measued, as previously described (20), by a modification of Dole's method. Tolbutamide interferes with the titration of the fatty acids (8, 22), and the elevations of plasma FFA observed in man a few minutes after tolbutamide injection (23, 24) appear to be due to the titration of this acidic compound (22). Consequently a study was made of the interference of tolbutamide with the titration of plasma FFA. Ten solutions containing known amounts of tolbutamide sodium (0.03–2.0 mg, in 1 ml of H_2O) were extracted with Dole's extraction mixture following the same procedure as with the plasma samples. Before titration the absorbance of the washed heptane phase, at 228 mμ, was measured in a Beckman D.B. spectrophotometer (25) using heptane as a blank. The heptane phase was then titrated and the titration figures were expressed as milliequivalents of FFA per liter of original solution. The calculated FFA value was closely correlated with the absorbance at 228 mμ ($r = +0.997$). With the data of three such experiments the regression equation $y = 0.7317\ x - 0.0194$ was calculated to predict the apparent amount of FFA, as milliequivalents per liter (y), corresponding to a given absorbance at 228 mμ (x). The heptane extracts of plasma samples from six geese, 5 min after the injection of tolbutamide (15.0 mg/kg), showed mean absorbance of 0.040

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[2] Orinase R_x Diagnostic. The Upjohn Company, Kalamazoo, Michigan.

[3] Crystalline glucagon, lot 258-234 B-167-1, Eli Lilly and Co., Indianapolis, Indiana, kindly donated by Drs. W. W. Bromer and W. N. Shaw.

TABLE I. Effect of Intravenous Injection of Tolbutamide on the Plasma Free Fatty Acids and Blood Sugar of Geese, Ducks, and Owls (means ± SE).

Bird	Number	Tolbutamide (mg/kg)	C1[a]	C2[b]	Time after injection (min) 5	15	30	60	90	120
Free fatty acids (meq/liter)										
Geese	8	15.0	1.18 ±0.06	1.16 ±0.06	0.84[c] ±0.04	0.74[c] ±0.06	0.70[c] ±0.06	0.71[c] ±0.08	0.78[c] ±0.09	0.76[c] ±0.09
Ducks	8	15.0	0.79 ±0.08	0.84 ±0.09	0.60[c] ±0.08	0.50[c] ±0.07	0.46[c] ±0.06	0.53[c] ±0.04	—	—
Owls	6	15.0	0.50 ±0.09	0.53 ±0.07	0.54[d] ±0.06	0.50[d] ±0.08	0.45[d] ±0.09	0.49[d] ±0.12	—	—
Owls	6	30.0	0.59 ±0.10	0.56 ±0.11	0.57[d] ±0.12	0.58[d] ±0.14	0.41[d] ±0.09	0.40[d] ±0.08	—	—
Blood sugar (mg/100 ml)										
Geese	8	15.0	127 ±9	135 ±8	131[d] ±8	99[c] ±9	64[c] ±8	59[c] ±4	71[c] ±5	77[c] ±6
Ducks	8	15.0	123 ±6	124 ±3	118[d] ±7	90[c] ±5	55[c] ±6	53[c] ±5	—	—
Owls	6	15.0	248 ±10	249 ±12	233[d] ±24	245[d] ±20	245[d] ±19	251[d] ±18	—	—
Owls	6	30.0	210 ±11	216 ±10	221[d] ±9	219[d] ±8	207[d] ±14	201[d] ±22	—	—

[a] Control sample 30 min before tolbutamide injection.
[b] Control sample just before tolbutamide injection.
[c] Significantly different from mean of control values C_1 and C_2 ($p < 0.01$).
[d] Not significantly different from mean of control values C_1 and C_2 ($p > 0.05$).

± 0.011 above that of extracts from plasma of the same animals taken before tolbutamide injection. This value, according to the equation, corresponds to 0.0099 meq/liter of FFA. No increase of absorbance was noted in samples taken 15 min after tolbutamide or later. The standard error of measurement of goose and duck plasma FFA in 68 pairs of duplicates with a mean value of 1.44 meq/liter, by five different analysts in this Laboratory, was ± 0.026 meq/liter. Furthermore no significant difference in FFA value was found when titrating duck's plasma extracts before and after adding 0.2 mg of tolbutamide sodium to 1.0 ml of blood. This corresponds to the maximum plasma tolbutamide concentration expected after injection of 15.0 mg of tolbutamide/kg, assuming total blood volume equal to 75 ml/kg, and distribution of tolbutamide only in the plasma. Accordingly no correction for tolbutamide interference was considered necessary. The values reported have been calculated from the titration figures in the usual way.

Blood sugar concentration was measured with *o*-toluidine (26). Unless stated otherwise the significance of the differences reported was calculated by the *t* test for paired variates.

Results. Plasma FFA and BS values of geese, ducks, and owls before and after tolbutamide injection are presented in Table I. Tolbutamide (15.0 mg/kg) caused a marked decrease of FFA and BS in geese and ducks. Plasma FFA levels were significantly lower than control 5 min after the injection. No significant decrease of BS was observed until 15 min after injection. The maximum decrease of FFA (0.47 meq/liter ± 0.066, or 40% of control) occurred in geese at 30 min, and that of BS (72 mg/100 ml ± 6.2, or 55% of control) at 60 min after injection. Corresponding values for the ducks were 0.36 meq/liter ± 0.054 (44% of control) and 71

TABLE II. Effect of Tolbutamide Injection (15.0 mg/kg iv) [a] on Plasma FFA and BS of Geese and Ducks Receiving an Infusion of Glucagon (0.2 μg/kg/min) (Means ± SE).

Bird and number		Time of glucagon infusion (min)								
		0	15	30	45	50	60	75	90	120
Plasma free fatty acids (meq/liter)										
Geese (6)	Tolbutamide	1.18 ±0.07	2.48 ±0.24	2.47 ±0.24	2.48 ±0.21	2.21 ±0.24	2.18 ±0.19	2.20 ±0.21	2.28 ±0.20	2.29 ±0.37
Geese (6)	No tolbutamide	1.19 ±0.12	2.32 ±0.26	2.32 ±0.27	—	—	2.37 ±0.29	—	2.46 ±0.30	2.52 ±0.32
Ducks (5)	Tolbutamide	0.99 ±0.08	1.71 ±0.13	1.78 ±0.14	1.75 ±0.12	1.69 ±0.14	1.67 ±0.12	1.64 ±0.14	1.69 ±0.15	1.72 ±0.18
Blood sugar (mg/100 ml)										
Geese (6)	Tolbutamide	106 ±7	155 ±11	182 ±16	195 ±18	198 ±19	203 ±20	208 ±22	215 ±22	235 ±25
Geese (6)	No tolbutamide	112 ±6	181 ±28	218 ±36	—	—	259 ±48	—	276 ±47	296 ±45
Ducks (5)	Tolbutamide	116 ±9	148 ±9	162 ±16	170 ±24	177 ±27	178 ±28	178 ±31	179 ±35	187 ±39

[a] Tolbutamide injected just after 45 min blood sample.

mg/100 ml ± 32 (57% of control). Both plasma FFA and BS were, in geese, significantly lower than control 2 hr after tolbutamide injection. In an experiment with two geese (15.0 mg tolbutamide/kg) the plasma FFA did not return to preinjection level until 6 hr after injection. The plasma FFA level for four ducks was, on the average, 0.36 ± 0.09 meq/liter ($p = 0.03$) below control, 3 hr after the injection of 15.0 mg of tolbutamide/kg.

In contrast, the owls showed no significant change of either FFA or BS after tolbutamide (15.0 and 30.0 mg/kg). Similar negative results were obtained in another two owls injected with 60 mg of tolbutamide/kg. Table II shows the effect of injecting tolbutamide 45 min after beginning of a continuous infusion of glucagon (0.2 μg/kg/min). Injection of tolbutamide given during glucagon infusion caused in geese some decrease of plasma FFA. Mean decreases from FFA level before injection were 0.27 meq/liter ± 0.076 ($p = 0.015$) at 5 min and 0.28 meq/liter ± 0.10 ($p = 0.03$) at 30 min after injection. These values, although smaller, were not statistically different from those observed when tolbutamide was given alone ($p > 0.05$, t test for nonpaired variates). The decrease of plasma FFA at 30 min in the geese infused with glucagon was, however, only 11% of the level before the injection of tolbutamide.

Tolbutamide caused a very small and nonsignificant decrease of plasma FFA in ducks infused with glucagon. Decreases at 5 and 30 min after tolbutamide injection (50 and 75 min of glucagon infusion) were respectively 0.06 ± 0.06 and 0.11 ± 0.09 meq/liter ($p > 0.05$ for both differences).

The effect of glucagon given after the injection of tolbutamide was also tested. In two geese plasma FFA decreased from a mean control level of 0.85 to 0.59 meq/liter 30 min after the injection of tolbutamide (15.0 mg/kg). Infusion of glucagon (0.2 μg/kg/min), started 30 min after tolbutamide injection, elevated plasma FFA to a level of 1.68 meq/liter 30 min after the beginning of the infusion (1 hr after tolbutamide injection). The mean elevation above the preinfusion level caused by glucagon (1.09 meq/liter) compares with a mean elevation (1.13 meq/liter) observed at the same time of infusion in the six geese infused with glucagon alone reported in Table II. Since the plasma FFA level 1 hr after injection of tolbutamide is identical to the level 30 min

after the injection (Table I) this indicates that the effect of glucagon on the plasma FFA was not modified by the previous injection of tolbutamide.

Discussion. Injection of tolbutamide (15.0 mg/kg) caused a significant decrease of plasma FFA and BS in geese and ducks, but the owl showed no significant changes of either FFA or BS when injected with doses of tolbutamide up to 60 mg/kg. The BS changes observed in geese and ducks compare with those reported by other authors in these birds and in the domestic fowl (10–12). The BS changes developed slower than the FFA changes. The maximum decreases of BS, as percentage of the preinjection level, were, however, somewhat greater than those of FFA. The decrease of plasma FFA caused by tolbutamide in mammals has been explained as a consequence of insulin release (1, 2, 7), and it has been reported that tolbutamide produces an elevation of plasma insulin concentration in the duck (19). Since insulin does not lower plasma FFA levels in a number of birds (14–18), such an explanation does not apply to our observations in geese and ducks.

Stone *et al.* have reported that tolbutamide inhibits *in vitro* the lipolytic activity of rat adipose tissue and the lipolytic affect of glucagon on the isolated adipose tissue cells of the rat (8, 9, 27). Our observations, however, indicate that tolbutamide, at a dose which produced a significant decrease of plasma FFA in the intact goose and duck, does not cause a greater decrease of plasma FFA in these birds when injected during the infusion of glucagon at the rate of 0.2 μg/kg/min. Furthermore, the effect on plasma FFA levels of glucagon infusion at the submaximal dose of 0.2 μg/kg/min (21) was not affected by the previous injection of tolbutamide. It appears therefore that tolbutamide does not inhibit in birds the adipokinetic effect of glucagon *in vivo*.

It has been suggested that sulfonylureas inhibit the production (or release) of glucagon by the pancreas (28, 29), and Samols *et al.* (19) have recently reported a decrease of plasma glucagon levels after injection of tolbutamide (15.0 mg/kg) in ducks. Because of the remarkable adipokinetic effect of glucagon in birds (17, 18, 20, 21), it is conceivable that the decrease of plasma FFA produced by tolbutamide may be due to the suppression of glucagon release by the pancreas. However, the data reported by Samols *et al.* (19) indicate that plasma glucagon concentration returns to preinjection level 1 hr after injection. The plasma FFA level of our ducks, given the same dose of tolbutamide as that used by Samols *et al.*, was still significantly lower than control 3 hr after injection. The depression of plasma FFA levels produced by tolbutamide is clearly of longer duration than the decrease of plasma glucagon. In view of the speed of the FFA response to glucagon (20, 21), it seems unlikely that the sustained reduction of plasma FFA caused by tolbutamide can be explained solely by the changes in plasma glucagon concentration reported by Samols *et al.*

Summary. Intravenous injection of tolbutamide (15.0 mg/kg) caused a significant decrease of plasma FFA and BS concentration in geese and ducks, but not in owls. The decrease of plasma FFA was significant 5 min after the injection whereas the decrease of BS became significant first at 15 min of injection. The decrease of plasma FFA produced by tolbutamide in geese and ducks whose plasma FFA had been elevated by continuous infusion of glucagon (0.2 μg/kg/min) was not greater than that observed in intact birds injected with the same dose of the drug. Glucagon infusion in geese previously injected with tolbutamide caused an elevation of plasma FFA comparable to that observed when glucagon was infused at the same rate in intact birds. These results indicate that tolbutamide does not inhibit the adipokinetic effect of glucagon *in vivo*.

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1. Bierman, E. L., Roberts, T. N., and Dole, V. P., Proc. Soc. Exp. Biol. Med. **95**, 437 (1957).

2. Bellens, R., Acta Endocrinol. Copenhagen Suppl. 61 (1961).

3. Balasse, E., Malaisse, W., Franckson, J. R. M., and Conrad, V., Arch. Int. Pharmacodyn. Ther. **155**, 477 (1965).

4. Stone, D. B., and Brown, J. D., Diabetes **15**, 314 (1966).

5. Shima, K., and Foa, P. P., *in* "Tolbutamide . . . After Ten Years" (J. H. Butterfield and W. van Westering, eds.), p. 217. Excerpta Medica, Amsterdam (1967).

6. Ulrich, E., Frank, I., Kujat, G., Miller, G., and Serge, K., Endokrinologie **54**, 442 (1969).

7. Conrad, V., *in* "Tolbutamide . . . After Ten Years" (J. H. Butterfield and W. van Westering, eds.), p. 189. Excerpta Medica, Amsterdam (1967).

8. Stone, D. B., Brown, J. D., and Cox, C. P., Amer. J. Physiol. **210**, 26 (1966).

9. Brown, J. D., Stone, D. B, and Steele, A. A., Metabolism **18**, 926 (1969).

10. Hazelwood, R. L., and Lorenz, F. W., Endocrinology **61**, 520 (1957).

11. Mirsky, I. A., and Gittelson, S., Endocrinology **61**, 148 (1957).

12. Mirsky, I. A., and Gittelson, S., Endocrinology **63**, 345 (1958).

13. Snedecor, J. G., Matthews, H., and MacGrath, W. B., Poultry Sci. **35**, 355 (1956).

14. Heald, P. J., McLachlan, P. M., and Rookledge, K. A., J. Endocrinol. **33**, 83 (1965).

15. Lepkovsky, S., Dimick, M. K., Furuta, F., Snapir, N., Park, R., Narita, N., and Komatsu, K., Endocrinology **81**, 1001 (1967).

16. Grande, F., Proc. Soc. Exp. Biol. Med. **130**, 711 (1969).

17. Grande, F., Proc. Soc. Exp. Biol. Med. **133**, 540 (1970).

18. Langslow, D. R., Butler, E. J., Hales, C. N., and Pearson, A. W., J. Endocrinol. **46**, 243 (1970).

19. Samols, E., Tyler, J. M., and Miahle, P., Lancet **1**, 174 (1969).

20. Grande, F., Proc. Soc. Exp. Biol. Med. **128**, 532 (1968).

21. Grande, F., and Prigge, W. F., Amer. J. Physiol. **218**, 1406 (1970).

22. Weenink, R. O., and Smith, R. B. W., J. Clin. Endocrinol. Metab. **29**, 604 (1969).

23. Gebbie, T., and Reid, J. D., Lancet **2**, 661 (1964).

24. Maingay, D., De Ruyter, H. A., Touber, J. L., Croughs, R. J. M., Schopman, W., and Lequin, R. M., Lancet **1**, 361 (1967).

25. Forist, A. A., Miller, W. L., Krake, J., and Struck, W. A., Proc. Soc. Exp. Biol. Med. **96**, 180 (1957).

26. Hyvärinen, A., and Nikkilä, E. A., Clin. Chim. Acta. **7**, 140 (1962).

27. Stone, D. B., and Brown, J. D., *in* "Tolbutamide . . . After Ten Years" (J. H. Butterfield and W. van Westering, eds.), p. 202. Excerpta Medica, Amsterdam (1967).

28. Anderson, G. E., Perfetto, A. J., Termine, C. M., and Monaco, R. R., Proc. Soc. Exp. Biol. Med. **92**, 340 (1956).

29. Izzo, J. L., Ann. N.Y. Acad. Sci. **74**, 582 (1959).

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