Physiological Responses to Blood Collection Methods in Rats[1] (36041)

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Manipulative procedures incident to physiological data collections, despite caution used, often excite animals. Such excitement generally releases epinephrine, which directly alters blood sugar in mammals (1–4) through its hyperglycemic action on the liver (5, 6). As recent evidence (7, 8) suggests that even the method of collecting blood is sufficient stress to acutely influence adenohypophyseal hormones in rats, it is likely that plasma glucose and liver glycogen also may be influenced by blood collecting methods. These considerations led to the present investigation.

Materials and Methods. Animals. The 236 animals used in this study were adult male Sprague-Dawley rats, weighing 300–450 g each. Prior to the collection of data, the animals were exposed, in series, for at least 3 weeks, in a controlled environment room (Sherer-Gillett, CER 810) to artificial days of 12 hr of warm (27 ± 1°) and light followed by 12 hr of cold (16 ± 1°) and dark. The light period was always 0600 to 1800 hr; the dark, 1800 to 0600. Relative humidity was maintained at 40 ± 5% for all trials. All animals, except the fasted groups were on *ad libitum* feed (Purina Rat Chow) and water.

All experimental trials were between 1400–1500 hr—when rat activity and food and water intake appear to be minimum (9, 10).

Animal weights were obtained on a top-loading balance (Mettler, Model P1200) after euthanasia in all but the decapitated-preweighed group. In this group, a preweight —taken 24 hr prior to the experimental trial —was used. In the decapitated-weighed group, the duration of the handling procedure included weighing of the animal.

Liver weights (wet and dry) were obtained with an analytical balance (Mettler, Model H10T). Liver glycogen was measured on fractional samples (0.5 g of the caudate lobe) of the liver and results were calculated as a percentage of the whole liver according to a modified Pflüger method (11).

Method of blood collection. Two blood sampling methods were used in this study: heart puncture and exsanguination (Table I). In the former method, the duration of animal handling included the time between removing the animal from its cage through filling of the heparinized syringe. In the latter method, cervical vessel blood was collected immediately after decapitation in heparinized centrifuge tubes.

After the blood sample was obtained by heart puncture, the restrained-manual group was given an overdose of iv pentobarbital sodium. In other groups, euthanasia was by anesthesia, a blow to the head, or decapitation. Ether and chloroform anesthesia were administered by placing the rats in a small jar completely saturated with the anesthetic to induce rapidly a surgical level of anesthesia. Decapitation was performed with a Model 130 decapitator (Harvard Apparatus).

Following the collection of blood and the filling of the microcapillary pipettes, the plasma was immediately separated from the blood cells and frozen until glucose determinations were made, usually within 48 hr. Plasma glucose measurements were according to a modified Raabo and Terkildsen (12) glucose oxidase method (Sigma Chemical Company) using nondeproteinized plasma. Packed cell volumes were measured according to the microcapillary hematocrit method. Plasma protein was measured with a hand protometer

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TABLE I. Duration of Handling Procedure and Method of Collecting Blood from Adult Male Sprague-Dawley Rats.

Group	Sampling method[a]	N[b]	Duration (min)[c]
Restrained, rat board	Ex S	9	3.95 ± 0.39
Anesthetized, ether	HP	18	3.80 ± 0.32
chloroform	HP	9	2.90 ± 0.20
Stunned, fasted 24 hr	HP	13	1.99 ± 0.22
Stunned	HP	13	1.99 ± 0.22
Restrained, manual	HP	9	1.43 ± 0.36
Decapitated, weighed	Ex S	4	1.37 ± 0.09
fasted 24 hr	Ex S	4	0.90 ± 0.06
preweighed	Ex S	4	0.90 ± 0.06

[a] Ex S = exsanguination; HP = heart puncture.
[b] N = number of animals per group.
[c] Mean ± SE.

(National Instrument Company).

Results and Discussion. The influence of animal handling on plasma glucose is apparent from Table II data. Plasma glucose values, in order of descending magnitude, appear to be directly related to handling time. However, these results may be related to other factors.

These values are generally higher than those previously reported for rat plasma (13, 14). For example, the glucose value of the stunned-fasted group is 111 ± 3.4 mg/100 ml compared with 93 mg/100 ml (14), 104 mg/100 ml (15), and 110 mg/100 ml (13) in nonfasted rats (13–15). The apparent discrepancy is thought to result from the method of determining glucose. On the other hand, the elevated plasma glucose values during ether and chloroform anesthesia probably resulted from the transient hyperglycemic effect of those two substances with decreased use of carbohydrate, mobilization of glycogen as glucose during the period of increased epinephrine production, and depressed insulin production (16).

The increased values for the restrained-rat board group (Table II) over the restrained-manual group may be related primarily to duration of the former treatment—about 3 times longer than the latter treatment. However, values for the decapitated-preweighed and the decapitated-weighed groups did not differ significantly even though the latter required 50% more handling.

Blood glucose values were higher in all decapitated groups compared to restrained-manual and stunned groups. This probably resulted, first, from the massive sympathetic discharge with decapitation and the concomitant epinephrine release at the sympathetic nerve endings, including those in the liver (16–18) and, second, from exsanguination not allowing the blood to enter general systemic circulation freely so the glucose exchange with interstitial fluid could occur.

Correlation of plasma glucose with handling time was $r = 0.703$, with 0.707 significant at the 5% level—a relationship described by the

TABLE II. Influence of Animal Handling on Plasma Glucose.

Group	N[a]	Plasma glucose[b] (mg/100 ml)	% Difference[c]
Anesthetized, ether	40	165.3 ± 2.7[d]	+35.2
chloroform	9	160.0 ± 5.2[d]	+30.8
Restrained, rat board	9	149.6 ± 6.8[e]	+22.3
Decapitated, preweighed	19	141.2 ± 2.2[ef]	+15.4
weighed	61	135.9 ± 1.3[f]	+11.1
fasted 24 hr	9	124.3 ± 1.6[g]	+1.6
Restrained, manual (control)	40	122.3 ± 2.5[g]	0
Stunned	38	121.5 ± 2.4[g]	−0.6
Stunned, fasted 24 hr	11	111.1 ± 3.4[h]	−9.2

[a] N = number of animals per group.
[b] Mean ± SE; those with different superscripts *defgh* differ significantly ($p < .05$).
[c] Percentage difference = 100 [(experimental—control)/control].

TABLE III. Influence of Animal Handling on Liver Glycogen.

Group	N^a	Liver glycogen[b] (% of liver wt)	% Difference[c]
Decapitated, preweighed (control)	19	3.16 ± 0.17^d	0
weighed	61	2.29 ± 0.06^e	—27.5
Stunned	38	2.03 ± 0.11^f	—35.8
Anesthetized, chloroform	9	2.01 ± 0.09^f	—36.4
Restrained, rat board	9	1.90 ± 0.10^f	—39.9
Anesthetized, ether	40	1.80 ± 0.11^f	—43.0
Restrained, manual	40	1.04 ± 0.10^g	—67.1
Decapitated, fasted 24 hr	9	0.47 ± 0.13^h	—85.1
Stunned, fasted 24 hr	11	0.34 ± 0.10^h	—89.2

[a] N = number of animals per group.
[b] Mean ± SE; those with different superscripts *defgh* differ significantly ($p < .05$).
[c] Percentage difference = 100 [(experimental—control)/control].

equation $y = 119.5 + 9.76x$. In any event, about 49% of the increase in plasma glucose appears to be due to duration of handling time ($r^2 = 0.49$).

The influence of animal handling on liver glycogen is shown by data in Table III. The values are listed in order of descending magnitude, which differs from plasma glucose values—partially perhaps because decapitation and anesthesia affect liver glycogen less than plasma glucose. However, liver glycogen quite often is at or above control level after epinephrine is administered (19). Our values for liver glycogen agree with the normal range of 1.9–4.7% reported by others (13, 14, 20) except for the two fasted groups and the restrained-manual group.

The overdose of intravenous sodium pentobarbital may, in some way, account for the small percentage of liver glycogen in the manually restrained rats. However, dry weights of their livers, not shown, did not differ significantly from others. Excluding the two fasted groups for obvious reasons, it appears that liver glycogen and handling time are not significantly correlated although, generally, an increased handling time results in a decreased liver glycogen.

There were no significant differences in packed cell volume or plasma protein concentration between any of the experimental groups.

Conclusions. The results presented indicate that: (a) the method of obtaining a blood sample, including animal handling, can significantly affect the parameter being measured; and (b) duration of handling can affect plasma glucose and liver glycogen values.

1. Bearn, A. G., Billing, B., and Sherlock, S., J. Physiol. (London) **115**, 430 (1951).
2. Ambrus, J. L., Johnson, G. C., Chernick, W. S. and Harrison, J. W. E., J. Pharmacol. **113**, 2 (1955).
3. Lundsgaard, E., Nielsen, N. A., and Orskov, S. L., Skand. Arch. Physiol. **81**, 11 (1939).
4. Dickman, S. R., Wiest, W. G., and Eik-Nes, K., Amer. J. Physiol. **194**, 327 (1958).
5. Cori, G. T., Amer. J. Physiol. **94**, 557 (1930).
6. Vosburgh, C. H., and Richards, A. N., Amer. J. Physiol. **9**, 35 (1903).
7. Neill, J. D., Endocrinology **87**, 1192 (1970).
8. Grosvenor, C. E., McCann, S. M., and Naller, R., Endocrinology **76**, 883 (1965).
9. Besch, E. L., Aerosp. Med. **40**, 1111 (1969).
10. Besch, E. L., Aerosp. Med. **41**, 1145 (1970).
11. West, E. S., and Todd, W. R., "Textbook of Biochemistry," 3rd ed. Macmillan, New York (1963).
12. Raabo, E., and Terkildsen, T. G., Scand. J. Clin. Lab. Invest. **12**, 402 (1960).
13. Kmetec, E., and Heymann, W., Amer. J. Physiol. **207**, 1295 (1964).
14. Rubenstein, D., Amer. J. Physiol. **203**, 1033 (1962).
15. Altman, P. L., and Dittmer, D. S., "Environmental Biology." Fed. Amer. Soc. Exp. Biol., Bethesda, Md. (1966).
16. Brewster, W. R., Jr., and Bunker, J. P., Amer. J. Physiol. **171**, 37 (1952).
17. Himms, J., Pharmacol. Rev. **19**, 367 (1967).
18. Sutherland, E. W., and Rall, T. W., Pharmacol. Rev. **12**, 265 (1960).
19. Evans, C. L., Isai, C., and Young, F. G., J. Physiol. (London) **73**, 103 (1931).
20. Deuel, H. J., J. Biol. Chem. **123**, 257 (1938).

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