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Evaluation and control of variability in hormone-stimulated lipolysis in rat adipose tissue

SIR,—We use adrenaline-stimulated lipolysis in isolated rat epididymal fat pads to assay the antilipolytic property of various prostaglandins (Pike, Kupiecki & Weeks, 1967). For accurate assays and screening tests, small pieces of tissue have proved to be impractical because of the variation in the lipolytic response. This variation has been minimized (Schusterová, Krčíková & others, 1964; Carlson, 1965; Finger, Page & Feller, 1966) by distributing tissue from several rats among replicate vessels or by mincing the fat with scissors and using samples from the minced pool. We have extended and evaluated a method using finely chopped tissue pools to control variability between replicate samples whereby up to 30 replicates with 200 mg of tissue each may be used.

The distal portion of fat pads from 12 to 16 rats (male, 260 to 300 g) are removed under ether anaesthesia and incubated for 2 hr in about 125 ml of Krebs-Ringer bicarbonate solution (without glucose, with 3·2% albumin, always aerated with air 95% and carbon dioxide 5%. This pre-incubation allows basal lipolysis to become minimal (Vaughan, 1967). Each fat pad is cut into "cubes" with a McIlwain chopper (H. Mickle, Gomshall, Surrey) (McIlwain & Buddle, 1953), set for 0·73 mm thickness and 80 to 85 strokes/min. The chopped tissue is pooled in 75 ml of Krebs solution and mixed continuously using a magnetic stirrer. Any tissue not completely chopped is removed and the mixture is then centrifuged for 3 min at 1000 rev/min to separate shreds of filter paper from the chopper pad. The floating cake of chopped tissue is then divided among two or three 50 ml beakers and resuspended with stirring in 25 ml of Krebs solution at room temperature. Tissue may be kept for at least 2 hr without loss of lipolytic activity while stirred and aerated at room temperature, but no more than 30 min in a cake. Just before tissue samples are weighed, they are centrifuged again, and samples of 200 to 205 mg are

weighed and added to 10 ml of Krebs solution with 0·1 μg/ml adrenaline. The tissue is dispersed by brief mixing using a Vortex, Jr, and incubated for 60 min at 37° in a shaker set at 100–110 strokes/min. The fluid is aerated using a small polythene tube passed through a loose-fitting plastic cap with the tip placed just above the fluid. A specially designed incubation flask

TABLE 1. VARIANCE COMPONENTS IN ADRENALINE-STIMULATED LIPOLYSIS IN RAT ADIPOSE TISSUE

		Lipolysis rate μmole glycerol/g/hr							
		Tissue snips				Chopped tissue			
Expt*	No. rats	Means	Variation between	Component**	CV	Means	Variation between	Component	CV
		Control of within-rat variability by chopping tissue							
I	10	Right 4·75	Rats	2·30	33·4	1 9·66	Rats	10·29	34·0
		Left 4·35	Sides within-rats	0·59	16·9	2 9·22	Samples within-rats	0·38	6·6
		Mean 4·55	Snips	1·40	25·9	Mean 9·44	Glycerol†	0·30	5·8
		Control of between-rat variability by pooling chopped tissue							
II	10					Right 11·79	Rats	11·91	26·9
						Left 13·90	Sides within-rats	5·62	18·5
						Mean 12·84	Glycerol	0·31	4·4
						Pool 10·98	Samples	1·11	9·7
							Glycerol	0·37	5·6
		Comparison of variability between tissue snips and pooled chopped tissue							
III	9	Right 7·03	Rats	0·77	12·4	Pool 17·76	Samples	3·45	10·5
		Left 7·17	Sides within-rats	0·00	—		Glycerol	0·50	4·0
		Mean 7·10							

* See text for description. ** Variance component (s^2) associated with each source of variation (Bliss 1967). CV Coefficient of variation ($s/\bar{X} \times 100$). † Variance component between replicate glycero determinations.

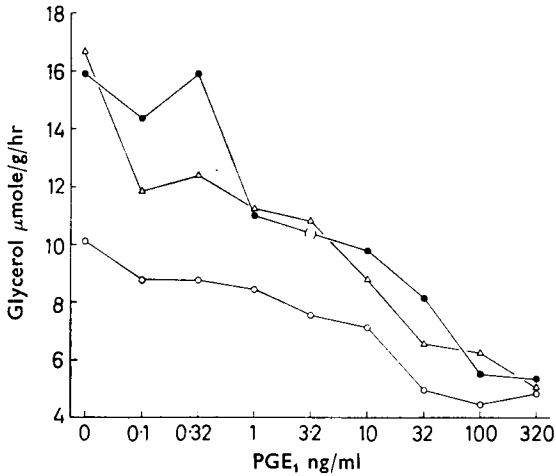

FIG. 1. Inhibition of adrenaline-induced lipolysis in chopped rat adipose tissue by prostaglandin E_1. Three replicate determinations, samples weighed consecutively from one tissue pool, the second and third determinations started 22 and 42 min after the first. Each point represents a single flask. Basal lipolytic rate without adrenaline was 0·87 μmole/g/hr. Entry ( ) is 18·0, discarded as due to experimental error. Replicate 1, ●; 2, △; 3, ○.

prevents tissue fragments from adhering to the sides.* Lipolysis is evaluated by glycerol release. We used the enzymic fluorometric method of Laurell & Tibbling (1966), slightly modified. Conventional tissue snips were 50 to 60 mg pieces cut from the distal tip and treated similarly using 1 ml of Krebs solution.

The three experiments in Table 1 demonstrate the variability in the lipolytic response and the degree to which it can be controlled. In the first experiment (I), two snips were cut from each pad and the remainder of both pads were chopped and divided between two flasks. Comparing the two techniques, the coefficient of variation between rats was the same, however that for snips within rats was 25·9 and that for chopped samples within rats was only 6·6. In the second experiment (II), each pad was chopped and the individual lipolysis rates and that of a tissue pool of all pads determined (7 flasks). The coefficient of variation between rats was 26·9, but was reduced to 9·7 between samples from the tissue pool. The third experiment (III) was to demonstrate, in tissue from the same animals, that both within-rat and between-rat variability was controlled. Snips were cut as in the first experiment, then chopped tissue from all rats was pooled (10 flasks). The within-rat coefficient of variation for snips was the same as in experiment I. Likewise, that between samples from the tissue pool was similar to experiment II. The variability from the glycerol assay was relatively small and there was no consistent difference between right and left pads.

It should be noted that in all experiments the lipolytic rate of chopped tissue was over twice that of tissue snips. Presumably, the greater surface to mass ratio permitted more efficient exchange of metabolites.

Fig. 1 illustrates three replicate concentration-effect curves for prostaglandin E_1 inhibition of lipolysis. Tissue samples for the replicate curves were weighed consecutively, without subdividing the pooled tissue. The first two curves are virtually identical, but the third showed less lipolytic activity. Control vessels from subsequent assays for antilipolytic activity demonstrated that subdivision and resuspension of the tissue prevented this loss of activity. Seven experiments were conducted within a two-week period. In each experiment the tissue was subdivided into two pools and two samples were tested from each pool. There was no difference in lipolytic rate of the two tissue pools, although the second was used 25 min after the first. The coefficient of variation for replicate samples was 7·7.

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* Details of the flask and of the laboratory procedure may be obtained *direct* from the authors.

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