protein per gel for fetuin and ovalbumin, respectively. Amounts as low as 0.2 µg of fetuin and 0.7 µg ovalbumin can be conveniently quantitated using the high-sensitivity setting of the densitometer Thus, within these limits of linear relationship, standard curves covering various ranges of protein concentration can be plotted.

When whole human serum was electrophoresed and stained, a linear relationship between the amount of protein applied on the gel and the peak area was observed for all the glycoprotein bands (within certain limits for individual bands). On the other hand, a number of serum proteins (stained by Coomassie blue) did not stain with this technique (fig. 2). Also in a sample of CNS myelin proteins only the bands representing glycoproteins were stained. Other proteins, without glycosyl residues, were not stained although they were present in much higher concentrations in the sample. Thus, although this study did not determine the chemistry of the alcohol-dependent reaction, it may be tentatively concluded that the chromophore binds specifically and stoichiometrically to glycoproteins.

The simple and rapid procedure described here offers remarkably improved sensitivity for detection and quantitation of glycoproteins following their electrophoretic separation on polyacrylamide gels.

The comparison of 2 methods for glycoprotein staining in polyacrylamide gels. The gels were loaded with either 32.8 µg of fetuin or 65.5 µg of ovalbumin. The figures represent average values and SD from 4 experiments

Method	Peak area (cm^2)	
	Fetuin	Ovalbumin
Original technique of Matthieu and Quarles	3.6 ± 0.3	2.1 ± 0.2
Modified technique	64.3 ± 3.1	45.3 ± 4.5

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Harvesting large amounts of Drosophila embryos at precisely defined stages

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Summary. A simple method is described which makes it possible to eliminate the retained eggs of *Drosophila melanogaster* and to harvest a large amount of embryos at a defined stage of development. It is based upon carbon dioxide anesthesia, which brings about the expulsion of eggs remaining in the uterus.

One of the problems when working with *Drosophila* embryos is the difficulty of harvesting large amounts of embryos at precisely defined early stages of development. This is due to the fact that some females do not lay recently fertilized eggs, but retain them for a variable time[1,2], so that the age-span of collected embryos does not correspond exactly to the laying period because there are 10–15% of older embryos. Since mitotic cycles at early stages are very rapid (10–60 min^{1-3}), even a modest contamination by older embryos creates a significant proportion of cells or nuclei from stages more advanced than that of the desired sample[4].

Several methods have been proposed to avoid the effects of egg retention, such as collecting the eggs at a desired stage one at a time[3,5] or obtaining enough virgin females and allowing them to be fertilized just after the start of laying[4,6]. These arduous methods do not permit the collection of the large amounts of embryos needed for certain purposes, especially molecular studies. A light-dark cycle causes a burst of egg laying during the light to dark transition[2], producing a certain degree of homogeneity, but only once a day, so that this method necessitates the rearing of very large populations. In this paper we describe a method of harvesting large amounts of *D. melanogaster* embryos with

Table 1. Percentage of embryos at each early stage when a defined stage reaches the maximum

Stages	Time at which the stages reach their maximum (min)															
	A				B				C				D			
	90	145	195	255	90	145	195	255	90	145	195	255	80	135	180	245
Syncytium	**84.3**	9.9	3.1	3.1	**91.1**	6.7	4.9	4.9	**99.6**	11.8	8.4	8.4	**100**	9.0	9.0	9.0
Syncytial blastoderm	4.2	**75.5**	5.2	2.1	6.2	**83.5**	0.9	0.5	0.4	**87.7**	4.8	1.1	0.0	**91.0**	3.4	2.1
Cellular blastoderm	4.2	6.8	**71.0**	1.0	0.9	7.1	**80.4**	2.2	0.0	0.4	**83.9**	1.0	0.0	0.0	**87.6**	0.0
Gastrula	7.3	7.8	17.7	**93.9**	1.8	2.7	13.8	**92.4**	0.0	0.0	2.9	**89.4**	0.0	0.0	0.0	**88.9**

Embryos harvested after a laying period reach successive stages, each at a reproducible time after the start of laying. This time is expressed in minutes. A, B, C and D are the conditions shown in the figure, and the homogeneity of the sample in a defined time is expressed by the percentage of embryos at each stage. CO_2 treatment before laying (C and D) clearly shows the elimination of the retention effect especially at a short laying interval (30 min) since contaminating embryos are unfertilized eggs or dead embryos from earlier stages.

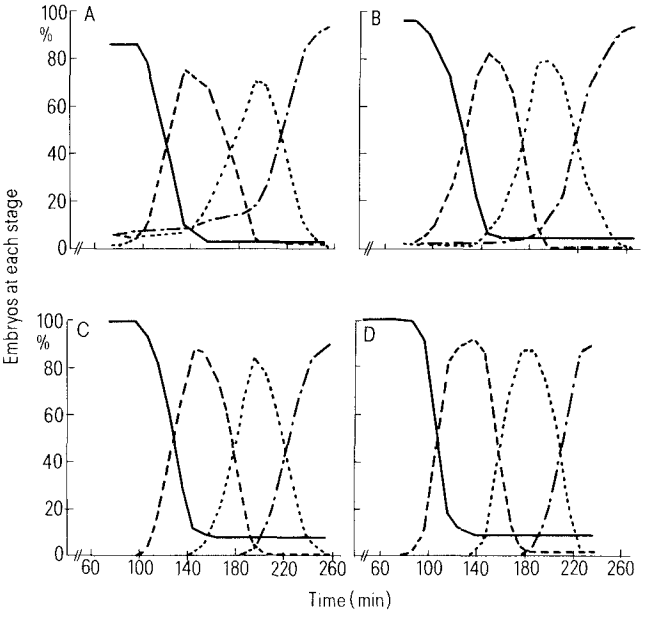
a very low retention effect (lower than 0.3%) and which allows us to obtain very homogeneous samples of embryonic stages from early ones (syncytium, blastoderm) to later ones. The method is based on muscular relaxation caused by carbon dioxide anesthesia, which provokes the expulsion of retained eggs[7].

A Berlin-K wild-type strain of *D. melanogaster* was used. The flies were obtained from mass cultures that had been reared in plexiglass cage populations maintained at 25 ± 1 °C. The flies were fed on 3.5% agar plates contained in trays (5 × 20 × 0.5 cm), coated with a cream point paste of yeast added to a solution of 30% sucrose, 20% glucose, 0.7% common salt and 4% propionic acid. The agar surface must be cut just before use, eliminating an upper coarse layer. CO_2 treatment was carried out in a laying cage consisting of a plexiglass cylinder 9 cm in diameter and 25 cm long, bearing a sleeve adjusted to fit the open end. A knot serves as a closure and the cloth allows transpiration. Along the lower side of the laying cage a 6 cm wide strip of filter paper is fixed; the anesthetized flies must be placed on this to prevent their sticking to the walls.

To harvest highly synchronized eggs, 4000–5000 flies, 4–8 days old, were placed in a laying cage, then a 40-l/h current of CO_2 was supplied to the cage for 5 min, taking care to keep anesthetized flies on the filter paper. The next steps were to eliminate CO_2 by means of an air current provided for 10 min and then to wait a further 10 min. During this 20-min period, the flies recuperated and managed to free the expelled eggs, which adhered to the filter paper and wall. Next, a tray with a thin coating of food was placed in the laying cage and the cage put in the dark. We considered this moment as zero time, starting the laying time which in our experiments was 30 min or 1 h. To collect eggs, the yeast paste was removed with a spatula and placed in a plexiglass cylinder one end of which was covered with a 250-mesh screen. The eggs were washed thoroughly with 0.7% NaCl, 0.1% Triton X-100.

When laying time was terminated, all the embryos collected were expected to be of the same stage and age (± 15 min or ± 30 min, depending on laying interval). In order to keep watch on the stages of embryonic development, a sample of collected eggs was dechorionated by immersion in 20% sodium hypochlorite (Carlo Erba) for 2 min[8] and rinsed with 0.7% NaCl. The dechorionated eggs were distributed in wells of microtest plates containing 0.7% NaCl, 0.1% Triton X-100. The embryos were observed by transmitted light at 100 times magnification with a dissection microscope[9]. Scores were recorded of each stage, every 10 min, starting 15 min after the end of laying time. The size of the samples was about 200 embryos. Samples of more than 1000 embryos were scored 75–85 min after the start of laying in order to have more accurate low retention values. In the experiments with successive layings, the amount of deposited eggs was measured by weighing the collected eggs before dechorionizing. All procedures were performed at 25 ± 1 °C in a thermostat-controlled room.

By means of embryo inspection we were able easily to distinguish 4 early stages: 1. *syncytium:* nuclear multiplication stage and embryo shortening within the vitelline membrane, 2. *syncytial blastoderm:* migration of nuclei to the surface of the egg, protrusion of pole cells and cell membrane extension inwards between nuclei, 3. *cellular blastoderm:* cell membrane progression until cellularization and the inception of infolding of the ventral furrow, and 4. *gastrula:* commencement of gastrulation, thickening of the blastoderm layer, invagination of the posterior midgut rudiment. All later stages found when the effect of retention was high, were scored as being at the gastrula stage.



Development of samples of *Drosophila* embryos at 25 °C. Dechorionated eggs were examined at 10 min intervals and individually assigned to one of 4 stages, viz.: syncytium (——), syncytial blastoderm (– – – –), cellular blastoderm (– – – –), and gastrula (– – – –). *A* Adult flies directly provided from mass cultures were allowed to lay for 1 h. We can observe a maximum for each stage but there is an overlapping between the different peaks of stages, due to the effect of egg retention before the start of laying. *B* Embryos harvested from a further laying of A. The retention effect is partially eliminated because many of the retained eggs were laid during the previous laying. Overlapping is extensive but lower than in A. *C* Flies were anesthetized 5 min with CO_2 and allowed to recuperate for 20 min and to proceed to 1 h of laying. With this procedure there is a regular replacement of stages, the only overlapping being caused by unfertilized eggs or dead embryos. *D* The same as in C, but the laying time was 30 min. Shortening the laying period can logically be expected to improve the result, because there is less difference in development between the first and the last eggs laid.

Table 2. Yield and effect of retention in 4 consecutive layings

	I					II				
Laying	Weight (g)	S	SB	CB	G	Weight (g)	S	SB	CB	G
1st	0.5	99.8	0.0	0.2	0.0	0.5	99.7	0.2	0.0	0.1
2nd	0.9	97.0	3.0	0.0	0.0	0.2	100	0.0	0.0	0.0
3rd	0.6	95.5	2.9	1.6	0.0	0.1	100	0.0	0.0	0.0
4th	0.3	94.1	3.4	1.6	0.9	0.15	100	0.0	0.0	0.0

S, syncytium; SB, syncytial blastoderm; CB, cellular blastoderm; G, gastrula. The table shows egg weight in g and the percentage of embryos at each stage in samples harvested just after 1 h of laying. In I, flies were CO_2 anesthetized before a series of 4 consecutive laying periods, in II before each consecutive laying period. Successive layings after only 1 anesthesia show that the retention effect appears progressively in the form of embryos in more advanced stages, but repetition of anesthesia before each laying period shows that the retention effect is completely eliminated. Embryos harvested from several laying processes may be grouped to accumulate embryos at a defined stage.

The figure shows embryonic development depending upon different laying conditions. Graphs A and B show the development of samples collected after 1 h of laying. Graphs C and D show samples after 1 h and 30 min laying, respectively, but with previous CO_2 treatment. Sample A was obtained after 24 h without rechanging the food and sample B after having discarded a former laying of 1 h. We can observe in graph A an overlapping between peaks of different stages. At 90 min after the start of the laying, when all the embryos should be in the syncytial stage, there are embryos at cellular blastoderm or gastrula stages, due to the retention effect. This effect is very variable; in some cases we observed up to 20% retention, mainly embryos older than gastrula. Sample B shows a lower retention effect indicating that the food conditions are an important factor.

The development graphs after CO_2 treatment (C and D) show sharper peaks which become free of embryos from later stages. The number of retained eggs in scores of 1000 embryos varies between 0 and 3 (the presence of these eggs may be fortuitous in part, because we have observed sometimes, just before the start of the laying period, flies bearing an egg adhering to the body). The treatment increases the amount of non-fertilized eggs about 5% over the control, thus the plateau of syncytium mortality seems to be higher. The different slopes and shifts of maxima are due to laying time, achieving good synchronization with 30-min laying. However, the number of eggs collected is less than half of the amount collected in a 60-min laying.

The reproducibility allows the harvesting of embryos of a defined stage merely by waiting long enough to reach the maximum of a stage after having removed the tray from the laying cage. The time for each stage in minutes after zero time for a 1-h laying is syncytium 90, syncytial blastoderm 145, cellular blastoderm 195, gastrula 255. For 30 min laying the times are syncytium 80, syncytial blastoderm 135, cellular blastoderm 180, gastrula 245.

The degree of synchronization achieved by this method is displayed in table 1, which shows the stage composition when a stage reaches the maximum. In the boxes there are the embryo percentages at their expected respective stages. It can be seen that the CO_2 treatment (C and D) eliminates the retention effect, therefore the fraction of more developed embryos becomes null. Non-fertilized eggs cause an increase of the fraction of syncytial embryos. The advantage of this method is clearly seen if we consider, for instance, the syncytial blastoderm (145 min) from B. Considering the mean number of nuclei per embryo of 120 for syncytium, 1440 for syncytial blastoderm, 6000 for cellular blastoderm and 12,000 for gastrula[3], the 145 min stage with 83.5% of syncytial blastoderm embryos, has in fact 38.2% of nuclei or cells from later stages.

Harvesting a large amount of eggs depends upon the number of flies per laying cage (more than 5000 flies is not useful) and the number of laying cages used. With this simple method of CO_2 treatment it is possible to use several laying cages simultaneously. In order to optimize the treatment, we have analyzed 4 consecutive layings after 1 anesthesia. The results are shown in table 2, which also shows the amount in g of eggs collected from each laying period. As should be expected, in the 2nd batch the retention effect begins to appear. The amount of eggs collected almost doubles in the 2nd batch but diminishes in subsequent batches. An alternative way is to repeat the CO_2 anesthesia before each laying period. Table 2 shows that the retention effect disappears absolutely in this way but the number of eggs decreases drastically. Therefore, to obtain a high yield, eggs may be collected twice after anesthesia but at the expense of homogeneity.

In conclusion, CO_2 anesthesia provides a simple method for harvesting large amounts of *D. melanogaster* embryos in the same developmental stage.

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A simple, less stressful rat restrainer[1]

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Summary. The contruction of a simple, inexpensive animal restrainer is described. Data are given showing its utility in analgesia studies.

For many pharmacological experiments it is necessary to restrict the motion of small animals such as the rat. This was the case for our analgesia experiments in which the rat tail-flick method was employed. The most commonly available restrainers for small animals are plastic or Plexiglas® cylinders that fit firmly about the animal. These units, while effective for immobilization, cause animals to become somewhat agitated while they are being persuaded to enter the restrainers. The effect of restraint in inducing some degree of analgesia in rats has been documented[2]. Hence, the use of the plastic tube type restrainer may lead to variable results due to the presence of this additional parameter. In this communication we describe the construction of a simple, yet highly effective cloth rat restrainer. A comparison between the tail-flick latencies obtained with a commercial plastic restrainer and the cloth restrainer is presented.

Materials and methods. Construction. The construction of