decrement in all these fractions was observed in the hypothalamus. These results suggest that in diabetic rats the receptors for estradiol were decreased in both cytoplasmic and nuclear fractions.

Our results thus support the view of Gentry et al.[6], in that uptake of ^{3}H-estradiol was reduced in AP nuclei of diabetic rats, but we also demonstrated in this gland a cytosol receptor defective in number, in affinity for the steroid, and probably in its translocation step from cytoplasm into the nuclear compartment.

The impaired binding of estradiol in the AP may be involved in the abnormalities in reproductive function described in experimental diabetes[1-4]. It is known that estradiol action on the AP is necessary for the responsiveness of the gonadotrophs to LHRH[15], and along this line, Kirchick et al.[16] demonstrated that in diabetic rats, the preovulatory LH surge is lost, presumably due to a decreased sensitivity of the hypothalamus and the AP to estradiol. This in turn would explain the anovulation of female diabetic rats.

Although little has been contributed to the knowledge of the regulation of estradiol-receptor interaction at the level of the AP, this binding reaction may be modulated by the hypothalamus. This conclusion is based upon recent demonstrations by us that rats bearing hypothalamic lesions bound considerably less estradiol in the AP[8], which would suggest that diabetic animals had this feature in common with rats with median eminence destruction. It is possible, therefore, that a defective hypothalamus lies behind the receptor changes of the AP described in the present report. However, other pathogenic factors cannot be excluded, as the changes in diabetes are not exclusive for estradiol. Decrements in binding and response to androgens and corticosteroids by the brain and AP of diabetic animals have been demonstrated by others as well as by our own laboratory[5,10,17,18].

1 R.A. Chieri, O.H. Pivetta and V.G. Foglia, Fert. Steril. *20,* 661 (1969).
2 E.G. Shipley and K.S. Danley, Am. J. Physiol. *150,* 84 (1947).
3 V.G. Foglia, R.F. Borghelli, R.A. Chieri, E.L. Fernández Collazo, I. Spindler and O. Wesely, Diabetes *12,* 231 (1963).
4 F.T.Y. Liu, H.S. Lin and D.C. Johnson, Endocrinology *91,* 1172 (1972).
5 J.H. Denari and J. Rosner, Steroids Lipids Res. *3,* 151 (1972).
6 R.T. Gentry, G.N. Wade and J.D. Blaustein, Brain Res. *135,* 135 (1977).
7 A. Junod, A.E. Lambert, L. Orci, R. Pictet, A.E. Gonet and A.E. Renold, Proc. Soc. exp. Biol. Med. *126,* 201 (1967).
8 L. Weisenberg, A.F. De Nicola, M.C. Arakelian and C. Libertun, Endocrinology, in press (1980).
9 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193,* 265 (1951).
10 O. Fridman, V.G. Foglia and A.F. De Nicola, J. Steroid Biochem. *9,* 609 (1978).
11 B.S. McEwen and R.E. Zigmond, in: Methods in Neurochemistry, 1972.
12 N.J. Mc Clusky, C. Chaptal, I. Lieberburg and B.S. McEwen, Brain Res. *114,* 158 (1976).
13 A.F. De Nicola, I. Von Lawzewitsch, S.E. Kaplan and C. Libertun, J. natn Cancer Inst. *61,* 753 (1978).
14 J.H. Clark and E.J. Peck, Nature *260,* 635 (1976).
15 G. Fink, Br. med. Bull. *35,* 155 (1979).
16 H.J. Kirchick, P.L. Keyes and B.E. Frye, Endocrinology *102,* 1867 (1978).
17 A.F. De Nicola, O. Fridman, E.J. Del Castillo and V.G. Foglia, Horm. Metab. Res. *9,* 469 (1977).
18 B.E. Howland and E.J. Zebrowski, Horm. Metab. Res. *8,* 465 (1976).

PRO EXPERIMENTIS

A simple apparatus to investigate the orientation of walking insects

H.J. Dahmen

Lehrstuhl für Biokybernetik, Institut für Biologie II, Auf der Morgenstelle 28, D-7400 Tübingen (Federal Republic of Germany), 14 September 1979

Summary. A simple apparatus to record the track and angular position of walking insects without any servo-mechanism is described. Recordings of fast running *Cicindelae* are presented.

To investigate the orientation of walking insects towards external stimuli it is often desirable to hold the animals in a constant position relative to the environment. This can be achieved by constraining them on the vertex of a sphere while they are running. Usually one lets the animals run upon a sphere which is counterrotated by a servo-mechanism[1-3]. We have built an appartus which records the translatory component of the animals' movements without any servo-mechanism and yet restricts their locomotion very little. Other non-servo ball type machines have been reported[4,5] which turned out to be of limited use.

The basic idea is to make the ball very light in weight and to let it float in an air stream in such a way that the ball can be handled, turned and moved easily by the animal itself. Figure 1 shows the essential parts of the apparatus: the ball used is a hollow sphere of Styropor® (polystyrene foam) of reasonable dimensions for the animal. It swims in an 'air cup', a hollow hemisphere of a slightly larger diameter than the ball (difference about 2 mm). Through a small hole in the bottom of the cup a weak air stream enters and supports the ball with nearly no friction and keeps it off the inner surface of the hemisphere in a surprisingly stable position. Since the diameter of the 'air cup' is slightly larger than that of the ball the cross-section of the airstream enlarges from the inlet to the equator of the cup and, consequently, the stream velocity decreases rapidly. The ball can easily be supported by an extremely weak air stream which does not cause any detectable air current on top of the ball in addition to the air flow usually present in the laboratory. Owing to Bernoulli-forces, increasing the velocity of the air stream merely results in a small change of the ball's position in the cup, and the ball will be more rigidly fixed in its position.

Good hollow spheres can be produced by boiling Styropor balls (available in toy shops) in an accurately-made metal mould for 3 min to give them the shape of a sphere of well defined diameter. Afterwards they are cut into 2 halves using a thin hot wire and hollowed out by hand by means of a hot wire loop; the 2 hollow halves are then glued together by a 2-component glue. Balls of 2 different sizes

have been produced so far: 5 cm diameter for smaller animals and 10 cm diameter for larger ones. Much larger and smaller balls could also be supported in this way by a weak air stream provided they are spheres of reasonable precision.

A very light metal needle is glued to the prothorax of the animals by means of a colophonium and beeswax mixture. This can be done without anaesthetizing the animals. They tolerate the manipulation well as can be judged from their behaviour afterwards. In particular, they do not try to get rid of the needle. The metal needle slides in a vertical glass tube so that the animal is allowed to turn and to move its prothorax up and down but not to change its position relative to its surroundings. When it runs it rotates the ball underneath. It can easily be shown that the 'effective mass' the animal has to accelerate is equal to $^2/_3$ of the mass of the thin-walled hollow ball. By careful carving of the ball its mass could be lowered to 3 or 4 times that of the animal or even less.

The apparatus is suited for a wide range of orientation experiments. It can be used in 3 different ways: 1. without registration of the rotations of the ball, the animals are merely kept stationary in space in order to measure e.g. the movement of the head, antennae or legs; 2. with registration of the ball's rotation (i.e. recording the animal's track); 3. with registration of the animal's momentary angular position and its path. To record the ball's rotation the air

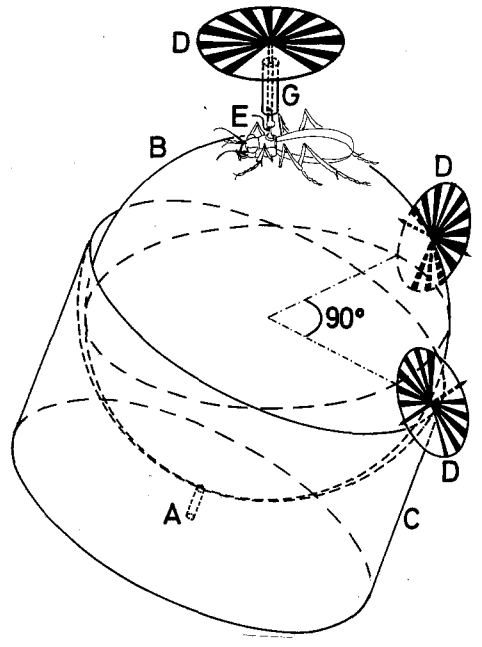


Fig. 1. Apparatus (without stimulus equipment): A = air supply, B = Styropor® ball, C = 'air cup', D = film disk with spoke pattern, E = elastic nylon thread, G = glass tube with needle.

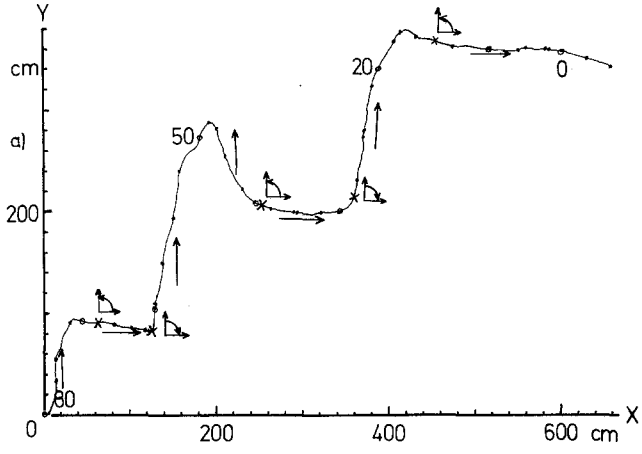
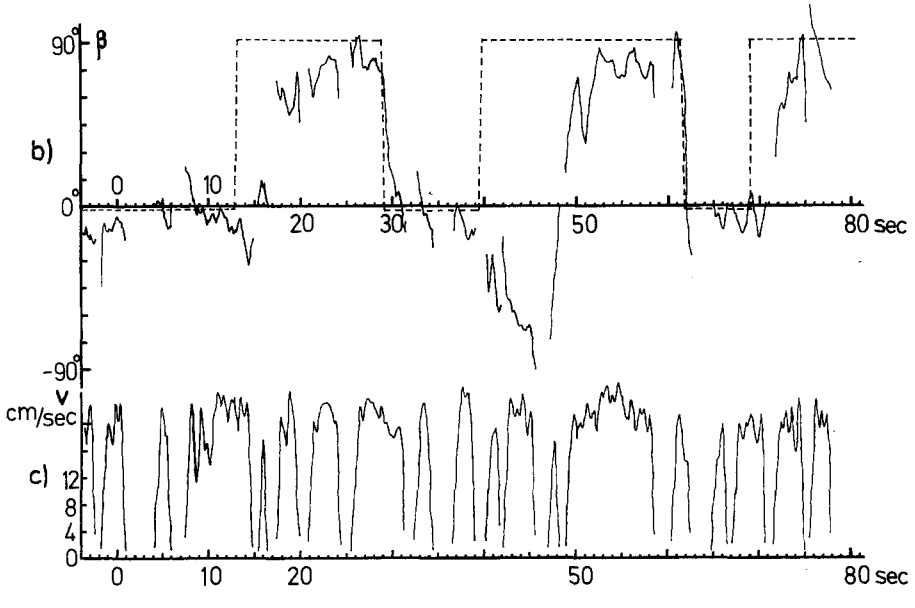


Fig. 2. Recordings of a track of *Cicindela silvatica* (80 sec of a 30-min track) are shown. The animal orientates towards a vertical white stripe 10° wide in azimuth on a black background. The angular position of the pattern is changed in steps. *a* shows the x-y-coordinates of the track. Long arrows indicate the direction of light impinging upon the animal from the stripe. Dots label 2-sec, circles 10-sec intervals. Crosses mark steplike changes of the angular position of the pattern, the size and direction of the step is given by the insets. *b* shows the angle β between the animals path and the negative x-axis as well as the angular position of the stripe (dashed line) versus time. The angle β is only measured, when the speed is above 1 cm/sec. *c* shows the speed v of the animal versus time. The locomotion of *Cicindela* is often interrupted. When it runs its speed is high and reaches about 16–25 cm/sec.



cup is tilted somewhat and 2 very light film discs with a spoke-like pattern press gently against the ball to keept it in position. The discs are glued to fine steel axes with tipped ends and supported by tip bearings normally used in alarm clocks. The 2 discs touch the ball in its equatorial plane at 90° to each other, as shown in figure 1. Each spoke pattern interrupts 2 light barriers. The interruptions are counted in order to measure the magnitude and direction of rotation of the discs and hence of the ball. The contents of the counters are read at programmable time intervals by a computer. In this way x- and y-coordinates of the animal's path can be recorded. The moment of inertia of the discs increases the effective mass of the ball the animal has to accelerate. The discs also restrict the ball to rotations around axes in the plane of the equator. A 3rd light spoke-pattern-disc, centred and fixed on top of the needle by a small drop of wax in combination with an additional light-barrier system allows for the recording of the angular position of the animal (figure 1).

The apparatus has been tested with 2 species of *Cicindela* and several other beetles. The tracks even of very fast running *Cicindelae* could be recorded successfully. The animals performed long runs (2–3 h). In most cases they did not show any obvious differences in their behaviour from that observed in the terrarium, provided that a very short nylon thread was inserted as an elastic joint between the needle and the prothorax, which allowed the animal to tilt and roll its prothorax to some extent. Figure 2 shows an 80-sec interval of a 30-min recording of a track of *Cicindela silvatica*. In the experiment the animal orientated towards a single vertical white stripe, 10° wide in azimuth on a black background. The track with its sudden starts and stops and a running speed up to 25 cm/sec could be recorded without difficulty (see figure 2, c).

1 K.G. Götz and Ch. Gambke, Zum Bewegungssehen des Mehlkäfers Tenebrio molitor. Kybernetik *4*, 225 (1968).
2 E. Kramer, The orientation of walking honeybees in odour fields with small concentration gradients. Physiol. Ent. *1*, 27 (1976).
3 D. Varjú, Stationary and dynamic responses during visual edge fixation by walking insects. Nature *225*, 330 (1975).
4 W.B. Kerfoot, Orientometer for study of insect behaviour. Science *162*, 477 (1968).
5 E. Buchner, Elementary movement detectors in an insect visual system. Biol. Cybernetics *24*, 85 (1976).

Methods for determining ploidy in amphibians: nucleolar number and erythrocyte size[1]

S.A. George and Michelle R. Lennartz[2]

Biology Department and Neuroscience Program, Amherst College, Amherst (MA 01002, USA), 20 August 1979

Summary. Diploid and triploid *Xenopus* can be easily and reliably distinguished by the size of their erythrocytes. This method has several advantages over other methods, such as counting metaphase chromosomes and counting nucleoli. One problem with the latter method is the reduction in cells with a full complement of nucleoli when regenerating tissue is used.

Triploid amphibians have been produced in the laboratory for a variety of purposes: e.g., to study nucleo-cytoplasmic interactions[3], to provide histologically identifiable tissue for embryonic transplants[4], and to investigate effects of cell size and number in the nervous system[5]. Since the methods for inducing triploidy (temperature or pressure shock to the early embryo[6,7]) are not 100% effective, resulting in some treated embryos being diploid (2N) rather than triploid (3N), it is essential to have reliable and efficient techniques for determining ploidy in these animals.

2 methods are commonly used to measure ploidy: counting chromosomes and counting nucleoli. There are disadvantages to both of these methods. Chromosome counts are laborious and, unless cells are grown in culture, somewhat imprecise because metaphase chromosomes in tissue such as the larval tail tend to be tangled and hard to count. Regarding nucleolar counts, it is assumed by most workers that the maximum number of nucleoli per nucleus in tail tip epithelial cells is equal to the ploidy of the animal. However, many cell types are known to deviate from this rule[8], and it is possible to confuse other organelles, such as the Barr body, with nucleoli[9]. Furthermore, caution must be exercised in the choice of tissue to be used in nucleolar counts. Regenerating tail tips are often used for counting chromosomes because regenerating tissue has a higher proportion of metaphase cells with visible chromosomes. It is often convenient to use the already prepared tail squash for counting nucleoli also. However, we have found a reduction in the average number of nucleoli per nucleus in regenerating tail tips compared to normal tail tips from the same animals, as has been reported previously for regenerating mammalian liver[10].

Analysis of nucleoli. Triploid *Xenopus laevis* were produced by subjecting fertilized eggs to 400 b of hydrostatic pressure for 6 min beginning 5 min after insemination. Tail tips were removed from 3 2N and 3 previously confirmed 3N tadpoles at stage 55, placed in distilled water and in aceto-orcein stain for 5 min each, and squashed on a slide under a coverslip. After 3 days, a 2nd squash was made from the same animals to provide regenerating tissue. The number of nucleoli per nucleus was counted in a total of 989 cells from these preparations. As can be seen in the table, the mean number of nucleoli was significantly lower in regenerating 3N tissue. Another way of looking at these data is to consider the proportions of cells with given numbers of nucleoli, as shown in figure 1. Note the decrease in the

Number of nucleoli per nucleus (mean ± SE) for diploid (2N) and triploid (3N) tail tip cells during ordinary growth (1st squash) and during regeneration (2nd squash)

	1st squash	2nd squash	t-test
2N	1.53 ± 0.04	1.47 ± 0.03	$p = 0.23$
3N	1.80 ± 0.5	1.57 ± 0.04	$p < 0.0001$