thyroid function is below physiological level. Further, this study indicated that disturbance in splenic cellular metabolism in hypothyroid mothers may partly be responsible for abnormal pregnancy performances. However, its role in controlling the reproductive phenomena, like that of the thymus, is yet to be established.

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PRO EXPERIMENTIS

An inexpensive and sensitive method for measuring and classifying activity in small animals

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Summary. A vibration-sensitive device which is useful in the quantification of activity in small animals is described. In addition to measurement ot total energy expenditure for activity, this transducer also allows identification of types of activity (sleeping, eating, active movement).

Quantification of activity of small animals such as rats and mice is often desirable in conjunction with the measurement of effects of various treatments. Several techniques for observing activity including activity wheels[1], force transducers[2], and photoelectric detectors[3,4] have been useful. The major drawbacks of these systems are either that they do not measure the total energy expenditure for movement (such as the activity wheel) or require delicate and not generally available apparatus. We propose a method which is extremely sensitive, allows classification of types of activity, and can be easily constructed from equipment available in most laboratories.

Apparatus. The transducer used to detect the animal's level and type of activity is essentially a vibration detector. It consists of a glass container partially filled with an electrolyte solution, such as 1% NaCl, with 2 partially immersed electrodes. The electrodes, which are simply lengths of wire of any conducting material, are inserted through 2 holes drilled in the cap of the glass container and sealed with epoxy. They should be separated by a distance great enough to prevent capillary action between them (approximately 0.5 cm) and positioned so that they are submerged 0.25–1.0 cm under the surface of the solution (figure 1). The signal from the transducer electrodes is fed to the inputs of a standard impedance bridge, amplified, and observed utilizing a pen recorder. In this system, it is possible to use long lengths of non-shielded cable without appreciable signal loss or interference.

The transducer is used to estimate activity by placing it on a flat surface on top of a spring-mounted shoebox type

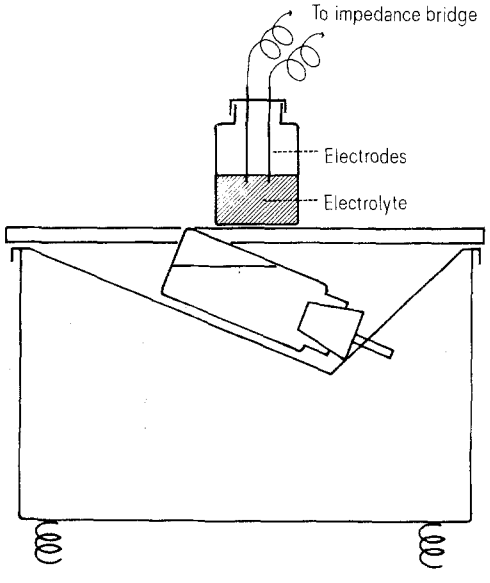

Fig. 1. Vibration-sensitive transducer placed on top of small animal cage on flat surface. Animal movements result in movement of the electrolyte solution and are detectable with an impedance bridge.

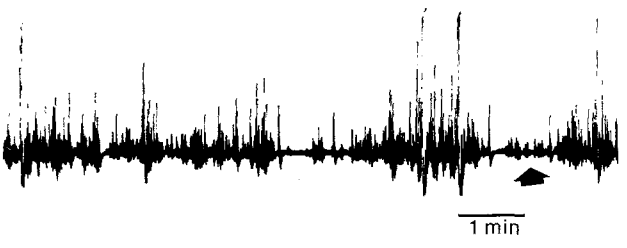

Fig. 2. Typical tracing obtained with the described device. Arrow indicates the type of pattern recorded while the animal is eating. Sleeping produces a flat line.

plastic cage of the design commonly used in the animal colony (19×25×15 cm). The type of spring mounting used will depend on the size and weight of the animal and the cage; for 20–40 g mice, we have found that 4 cm cubes of soft sponge rubber are effective. The fidelity of the apparatus is improved by using fairly rigid spring mounts.

Methods. The apparatus was tested by placing a laboratory mouse into the activity cage with food and water in the morning and allowing acclimatization throughout the day. At approximately 16.00 h the impedance bridge was calibrated to an arbitrary level using a built-in calibration source and the pen recorder (6 mm/min) was started. Recording was continued until the following morning. In this situation, the activity cage was in the mouse room and connected to the electronic equipment in a separate room at a distance of about 30 m. Therefore, measurements were made in the animal's normal environment during the dark phase of the light-dark cycle, that is, when mice are normally most active. Tracings obtained from 10 animals were measured to determine total level of activity and the portion of the recording period spent sleeping. Total activity measurement was accomplished by integrating the area under the 'curve' obtained (figure 2) either manually or by electronic integration of the original signal.

Results and discussion. Mice used to test the device showed the greatest level of activity during the first 2 h of the dark phase and became progressively less active during the night. Simultaneous observation of the animal and the tracing revealed that only periods of sleep produced a perfectly flat recording; even minimal preening activity was obvious. Measurement of sleeping periods demonstrated that the mice spent an average (±SEM) of 68.2±1.6% of the night in waking activity. Total level of activity, in arbitrary units, was 2032±216 (n=10).

It was also found that eating the pelleted food which was provided produced a characteristic tracing pattern which could be measured to provide an estimate of the time spent eating, a parameter which is useful in studies of obesity in rodents[5]. Further, it is possible to construct several transducers which provide essentially identical tracings. Therefore, animals from more than 1 treatment group can be evaluated during the same time period. Calibration of more than 1 transducer is accomplished by first adjusting 1 device as described above and then placing both transducers on a single cage and adjusting the amplitude of the 2nd device until both tracings are identical.

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An improved method for the isolation of eosinophilic leukocytes from normal human blood

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Summary. A relatively short and simple method for the isolation of the eosinophils from normal human blood is reported. With a recovery of about 45–55%, cell preparations, showing a degree of purity of 90–98%, are obtained. The isolated cells are morphologically intact and viable, as assessed by the trypan blue exclusion test and by active phagocytosis.

In connection with a better understanding of certain allergic reactions the study of the biochemical and the functional properties of isolated normal human eosinophils is of interest. Until recently all simple isolation methods used blood from patients with high eosinophilia (40–80%), the relatively diminished number of neutrophilic granulocytes facilitating the isolation of the eosinophils. However, recent investigations[2–4] have demonstrated that eosinophilia cells are, with respect of their biochemical and morphological properties (modified IgG receptors and enzyme content; vacuole formation and depleted granules), very different from those isolated from the blood of healthy subjects. The isolation of normal eosinophils is difficult because of the small density differences between human neutrophils and eosinophils. The method previously reported[5] for the isolation of the eosinophils from horse blood can not be used for human blood without modifications. Such a modified isolation method is described in this paper.

Material and methods. Isolation of human eosinophils: Cell sediments (about 3 l) of fresh citrated human blood, which, for various reasons, could not be otherwise used than for plasma production, were obtained from the local blood bank. These sediments, not older than 12 h, were again centrifuged (2500×g, 30 min, 4 °C; refrigerated 4 l-centrifuge). The plasma layer was aspirated, centrifuged until cell-free (Sorvall RC2B, 13,000×g, 20 min, 4 °C) and stored at 4 °C for further use (fraction 1). After aspiration of the buffy coat together with the upper fifth of the erythrocyte layer (fraction 2), the next 3 fifths of the red cell layer were removed and discarded. The remaining leukocyte-free red cell sediment, about 400 ml (fraction 3), was saved for further use. Fraction 2 was carefully mixed with half its own volume of plasma (ca. 200 ml) and centrifuged in a Sorvall RC2B centrifuge (rotor HS4, 4800×g, 30 min, 4 °C). The compact leucocyte layer was carefully and completely removed by aspiration. The erythrocyte layer (ca. 400 ml), containing the eosinophils, was mixed with 1.5 vol. of a solution containing 20% of the fraction 1 and 80% of a polyvinylpyrrolidone solution (60 g of polyvinylpyrrolidone K-60 (Fluka) and 10 g of NaCl in 1 l of H_2O). After spontaneous sedimentation of the red cells (30 min at room temperature) in a 1-l glass cylinder, the supernatant containing the eosinophils and some neutrophils was aspirated. The erythrocyte layer was resuspended in 1.5 vol. of fresh plasma-polyvinylpyrrolidone solution and resedimented. Thus, a 2nd portion of eosinophilrich supernatant could be aspirated. The combined supernatants (ca. 1.2 l) were centrifuged (250×g, 15 min). The cell sediment, resuspended in 40 ml of physiological saline, was divided into 4 equal portions, which were each separately superposed over cushions of red cells (24 ml of fraction 3 mixed with 6 ml of fraction 1) and centrifuged (Sorvall RC2B, rotor HB4,