

**Splenic enzyme systems as affected by hypothyroidism during pregnancy**J.N. Panda, J. Goswami and P.M. Rao<sup>1</sup>*Division of Physiology and Climatology, Indian Veterinary Research Institute, Izatnagar (U.P. 243122, India), 14 February 1979*

**Summary.** Influence of thyroid hormones on splenic activities and the role of the spleen during pregnancy were not known. The present study revealed that increased enzyme activities of spleen are associated with pregnancy, and hypothyroidism during gestation led to decreased activities, indicating that the thyroid gland influences them.

Spleen, thymus and other lymph glands are associated with the immunologic mechanisms of the body<sup>2</sup>. Thymus has been shown recently to be involved in the regulation of structure and function of the lymphoid tissue including spleen<sup>3</sup>. The role of splenic functions in the reproductive processes, like pregnancy, has been given little attention, although the thymus, a target organ of the hypophysis, has been shown to be very much related to sexual physiology<sup>4</sup>. Spleen cells completely corrected the reproductive disorders resulting from thymectomy<sup>5</sup>. There are reports about the inhibitory actions of uterine proteins of pregnancy on lymph glands other than spleen<sup>6,7</sup>. Further, the influence of thyroid hormone or pregnancy stress on splenic metabolism is not known. Hence the present study is aimed at probing into such unknown facts about this vital immunogenic organ.

**Materials and methods.** 50 adult female rats of approximately the same age and weight were maintained with food and water ad libitum. Estrus females were mated with proven males and the day one of pregnancy was determined by the presence of spermatozoa in the vaginal smear next morning. Bilateral thyroidectomy was performed surgically<sup>10</sup>. Spleens from individual animals of all the groups were collected on ice aseptically immediately after parturition and homogenized with ice-cold physiological (0.89%) saline. The enzymes and the protein in the homogenates were estimated by approved techniques<sup>10-12</sup>. Only animals showing complete absence of thyroid tissue, observed by histological studies of the surrounding area, were included in the experiment.

**Results and discussion.** The findings (table) show that thyroidectomy caused splenic alkaline and acid phosphatase and glutamic-oxaloacetic transaminase (GOT) activities significantly ( $p < 0.01$ ) lower, and glutamic-pyruvic transaminase (GPT) activities significantly ( $p < 0.05$ ) higher, than

those of the intact controls (group 1). But all the enzymes increased significantly in pregnant animals (group 3). When pregnant animals were made hypothyroid at different stages of gestation, all activities but acid phosphatase decreased significantly.

In all the hypothyroid groups parturition was difficult, gestation period lengthened, and the litter size and litter weight reduced as compared to the intact pregnant ones, similar to earlier findings<sup>13,14</sup>. Since these enzymes, mostly alkaline phosphatase and GOT, are concerned with cellular transfer mechanisms, nucleic acid metabolism, protein synthetic and above all, energy producing processes<sup>15</sup>, their increased levels are indicative of splenic hyperactivity. Spleen has both immunogenic and non-immunogenic functions which are possibly important in maintenance of pregnancy, firstly for increased antibody production that gets transferred to the foetus, and secondly for augmenting erythropoiesis both in the mother and the foetus by releasing  $Fe^{++}$ . Lymphoid tissue function is accelerated by thyroid hormone<sup>16</sup> but suppressed by progesterone, cortisone and uterine proteins<sup>6-8</sup>. Increase in enzyme activities in the spleen of intact pregnant rats as compared to the controls implied elevation in splenic metabolism perhaps due to the increased thyroid function caused by the stimulation of estrogens<sup>17</sup> during gestation.

It may also be possible that higher levels of splenic metabolism are necessary both for immuno-globin synthesis and to meet the other needs of pregnancy making this lymphoid organ conducive to reproduction, as steroids do not cause atrophy of spleen in any physiological condition. Thyroidectomy both in nonpregnant and pregnant animals caused reductions in enzyme activities; but duration of hypothyroidism during gestation failed to show any difference in the splenic function amongst the hypothyroid groups, indicating that the spleen is affected equally when the degree of

Effect of hypothyroidism during pregnancy on certain enzyme systems of the spleen of rats

Group	Treatment	No. of animals	m units (KA)/min/mg of sol. protein Acid phosphatase	Alkaline phosphatase	nmols of pyruvate formed min/mg sol. protein GOT	GPT
1	Control	6	10.15 ± 0.56	129.06 ± 13.15	35.73 ± 2.46	5.09 ± 0.87
2	Non-pregnant thyroidectomized (Thx)	6	5.45 ± 0.94	64.12 ± 4.33	26.41 ± 3.76	10.86 ± 2.04
3	Pregnant (Intact)	8	23.78 ± 3.14	157.83 ± 5.91	45.67 ± 2.56	29.59 ± 7.86
4	Pregnant Thx on 3rd day of pregnancy	6	28.91 ± 3.67	72.02 ± 9.19	33.44 ± 1.69	19.24 ± 1.45
5	Pregnant Thx on 6th day of pregnancy	8	26.77 ± 3.13	82.22 ± 8.16	30.34 ± 2.12	19.44 ± 5.08
6	Pregnant Thx on 8th day of pregnancy	6	24.40 ± 2.81	76.02 ± 6.53	29.07 ± 5.49	12.87 ± 2.80
7	Pregnant Thx on 10th day of pregnancy	6	23.37 ± 3.12	77.77 ± 4.74	25.23 ± 3.71	—
8	Pregnant Thx on 13th day of pregnancy	4	24.70 ± 3.26	80.51 ± 4.58	31.26 ± 5.19	17.20 ± 4.03
KA = King-Armstrong units.			1 vs 2 $p < 0.001$	1 vs 2 $p < 0.01$	1 vs 2 $p < 0.05$	1 vs 2 $p < 0.05$
Each figure is mean ± SE.			1 vs 3 $p < 0.001$	1 vs 3 $p < 0.05$	1 vs 3 $p < 0.01$	1 vs 3 $p < 0.01$
Students 't'-test has been used for test of significance.			3 vs others not significant	3 vs other $p < 0.001$	3 vs others $p < 0.01$	3 vs 6 $p < 0.05$

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 of 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<sup>1</sup>, force transducers<sup>2</sup>, and photoelectric detectors<sup>3,4</sup> 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

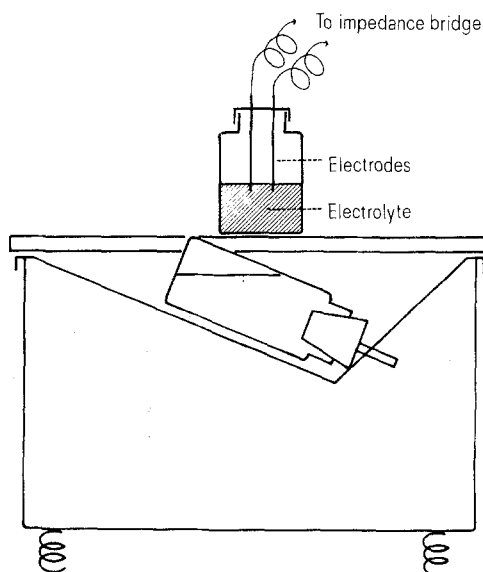


Fig. 1. Vibration-sensitive transducer placed on top of small animal cage on flat surface. Animal movements result in movement of the electrolyte solution and are detectable with an impedance bridge.

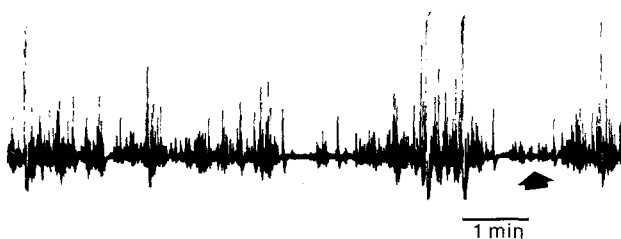


Fig. 2. Typical tracing obtained with the described device. Arrow indicates the type of pattern recorded while the animal is eating. Sleeping produces a flat line.