statistical procedures were performed using an electronic microcomputer (Olivetti No. P-652/ROM 01 – MLU-600).

Results and discussion. In the control group (0-time), 4.5% of the mitochondrial sections appeared to be labelled, indicating that, as in the zona fasciculata[2], also in the zona reticularis of intact rat adrenals about 5% of mitochondria were in 'S' phase. The CR of the mitochondrial compartment in the untreated rats remained unchanged during the first 3 days after the tracer injection, suggesting that in this period each labelled ('S' phase) mitochondrion divides into two organelles of which one is labelled. Thus we can consider that the fate of a pool of 3H-thymidine-labelled newly formed mitochondria was followed in the present experiment.

From the 3rd to the 15th day after the tracer injection, the CR of the mitochondrial compartment of untreated rats decreased in a semilogarithmic manner (figure, a) and the number of days in which the CR is reduced to a half, averaged 8.16 days. Since it is well established that DNA is an extremely stable molecule, which does not display appreciable turnover[10], we can reasonably assume that this parameter can be an estimate of the half-life of mitochondria.

In the ACTH-treated groups, the CR of the mitochondrial compartment did not show any significant change during the first 6 days after 3H-thymidine administration, and thereafter it decreased in a semilogarithmic manner (figure, b). The half-life of zona reticularis mitochondria of ACTH-treated rats was found to be significantly higher than in the untreated animals (12.13 vs 8.16; $p < 0.01$).

These findings suggest that the mechanism underlying the ACTH-elicited stimulation of the growth of the mitochondrial compartment from the rat adrenal zona reticularis involves not only hypertrophy and proliferation of the organelles[4], but also the slowing down of the degeneration rate of mitochondria as 'intact units'. Since the rate of degradation of adrenal mitochondrial proteins[11,12] and phospholipids[13] was demonstrated to be slower in the hypertrophic adrenals of ACTH-treated rats, we hypothesize that metabolic stabilization of the lipoproteic components of the mitochondrial membranes can be involved in the ACTH-induced increase in the half-life of mitochondria from the rat adrenal zona reticularis.

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Ovarian LDH Activity in Gonadotropin-Treated Immature Rats

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Summary. Lactate dehydrogenase (LDH) activity was studied in the ovaries of immature rats treated with pregnant mare serum gonadotropin (PMS). LDH activity increased sharply at 36 h after PMS injection in the ovarian tissue as well as in the blood. It was suggested that the increase of LDH activity in the ovary may be related to its increasing ability to secrete estrogen.

Lactate dehydrogenase (L-lactate: NAD oxidoreductase, E.C. 1.1.1.27) is a key factor of glycolysis and is mainly concerned with the restoration of depleted NAD during glycolysis. It was shown that ovaries exposed to a large dose of PMS had a marked increase in glucose-6-phosphate dehydrogenase[1]. In the immature rat, LH administration caused a marked increase in glucose uptake, lactic acid production and glucose oxidation[2]. The rate of lactic acid production by the ovaries from intact and hypophysectomized immature female rats was found to increase for 8 h after PMS administration[3], and then decline to the initial level by the 16th h after PMS injection. In the present investigation we are providing evidence that LDH activity can be induced in the immature rat ovaries by PMS administration.

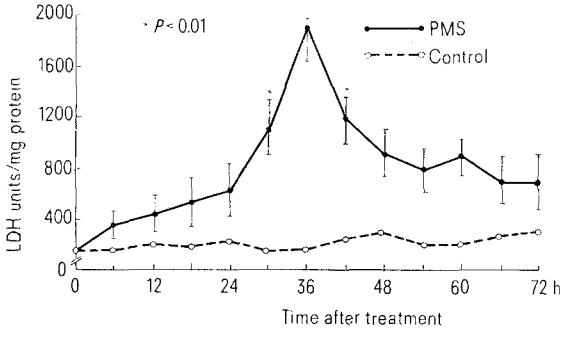

Fig. 1. Effect of 25 IU of PMS injected in the immature female rat (24 days old) on the LDH specific activity (enzyme unit/mg protein). Each point represents 6 animals and the standard errors are shown by the vertical lines.

Methods. Immature Holtzman strain female rats, 24 days old, were used in this experiment. They were kept in controlled light (14LD:10D). Feed and water were available to the animals ad libitum. To induce synchronous ovulation, groups of rats were given 25 IU PMS s.c. (Pregnant Mare Serum gonadotropin, Sigma Chemicals) at 900 h on the 24th day of age. Animals were sacrificed at 6.0-hour intervals and blood from trunk was collected. The occurrence of ovulation was determined by microscopic examination of the oviducts for ova.

After decapitation of the animals, the ovaries were removed immediately, dissected free from the bursa and extraneous tissues, and weighed. Routine assay of lactate dehydrogenase in tissue and blood was conducted as previously reported[4]. Protein concentrations were rou-

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tinely determined using the Biuret method. In conducting the experiment 6 animals were used for each time period and F-test analysis of variance was used to test for significance.

Results. All of the rats given 25 IU PMS on the 24th day of age ovulated during the night of the 26th. At 12.00 h on the 26th day, rats given PMS showed full uterine ballooning. At 24.00 h there was vaginal opening and ovulation as indicated by tubal ova. Although tubal ova were noted, no attempt was made to count them.

The specific activity of the enzyme (LDH units/mg protein) is shown in Figure 1. There was no significant difference ($p < 0.05$) between the 4 consecutive hours of 6, 12, 18 and 24, but there was an abrupt increase in the activity at 30 and 36 h (1,823 $\pm$ 287 and 1,168 $\pm$ 253 versus 172 $\pm$ 108 at 0 h; $p < 0.01$). The activity started to decline again at 42 h. The activity observed at 48 h was not significantly different ($p < 0.05$) from that observed at 48, 54, 60, 66 or 72 h. It is also clear from this Figure that there were no significant changes in LDH activity in the control.

The LDH activity (LDH units/ml) in the plasma of animals injected with PMS compared with animals which were not treated is presented in Figure 2. It is clear from this figure that there was an increase in LDH activity of blood at 36 h. This increase was significantly higher ($p < 0.01$) than the activity at the other hours (298 $\pm$ 46.7 versus 121 $\pm$ 18.5 at 0 h). Variations among the control show no significant variations along the period of the study.

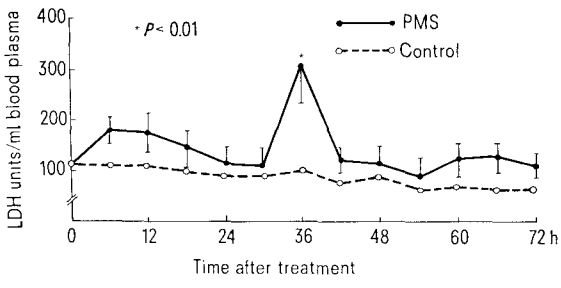

Fig. 2. Effect of 25 IU of PMS injected in the immature rat (24 days old) on blood LDH activity. Each point represents 6 animals and the standards errors are shown by the vertical lines.

Discussion. Studies on the molecular mechanisms of hormone action have indicated two general patterns. One pattern applies to the polypeptide hormones which act primarily through the activation of adenyl cyclase with the subsequent formation of cyclic AMP[5], and the second pattern applies to the steroid hormones which act primarily at the gene locus to initiate the synthesis of specific species of RNA[6]. The hormonal stimulation of LDH is not so easily delineated, but a conclusion might be drawn from the analogue results on estrogen level.

Recent studies indicated that estradiol levels reach maximum at 42–52 h after PMS injection in the immature rat and then decrease dramatically[7]. It is possible that lower levels of estrogen are able to induce LDH activity to its maximum, around 36 h. The direct effect of PMS in inducing LDH activity is unlikely. Human chorionic gonadotropin administration to the immature rats treated with PMS increased the amount of CAMP formed several fold in less than 1 h[8], which, if it were the mediator, should increase LDH activity immediately. Moreover, estrogen is known to act to induce mRNA for the formation of different enzymes[6].

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PRO EXPERIMENTIS

The Micro-Focal X-Ray Unit and its Application to Bio-Medical Research

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Summary. The micro-focal X-ray unit is a modified Cosslett and Nixon X-ray microscope of greater operational stability and flexibility. Its combination with a closed circuit television system provides a quick method of obtaining a point source of X-rays to examine the detailed structure of organs and biological specimens.

The term X-ray microscope has been used to describe apparatus which uses one or two electron lenses to focus an electron beam onto a target, from which the X-rays are emitted. The principles of this form of apparatus were first described in 1939[2,3]. The apparatus was developed by Cosslett and Nixon in the 1950's and early 1960's[4–8]. Since that time the machine has been used to study a wide variety of biological tissues[9]. However, the material examined has been restricted to small specimens; such as insects[4] or to specimens of a few millimetres thick, or thin sections[10]. This restricted application of the machine is due to the limitations in its desgin.

The micro-focal X-ray unit described herein is a modified X-ray microscope which has been designed to overcome the limitations of the Cosslett and Nixon machine. Also the application of the machine to bio-medical research has been increased by the incorporation of a closed circuit television system.

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