of antiserum being given on day 5, 10 ml on day 7 and 15 ml on day 9. These times were selected to coincide with the early stages of blastocyst development, which, we felt, were the stages most likely to be influenced by antisera to uterine protein. After the last injection the sows were retained in their respective pens and observed daily until about 110 days post coitum. At this time they were removed to the farrowing shed and kept until parturition had occurred, or it was obvious it was not going to occur. Number of young and lengths of gestation periods were recorded, as well as the time expired before the animal returned to estrus if she had not given birth.

Results. In the Ouchterlony tests for precipitating rabbit antibody against porcine uterine proteins, up to 8 bands can be detected against luteal phase protein whereas only 4 (at maximum) against follicular phase protein each with its corresponding antiserum. All share some serum antigens in common. Some of the bands appear with serum taken after 1 injection, others are not obvious until after 2 or 3 injections.

The results of the breeding experiments are shown in the Table. The 3 control animals had essentially normal litters within normal gestation periods, but the pregnancies of some members of the experimental classes were obviously influenced by the treatment. 2 of the 3 animals getting antiserum to luteal proteins did not give birth and did not recycle until 173 and 237 + days, respectively, after breeding. The 3rd animal had a normal pregnancy. Of the 3 animals getting antiserum to follicular protein 2 had normal gestation periods, but the 3rd one had a gestation period prolonged by 9 days. For comparison, it will be noted that in this herd, gestation does not normally vary by more than a few days from 113 days with an average of 7.2 piglets/litter.

Discussion. The one prolonged gestation period in a pig getting antiserum to follicular phase protein is highly unlikely by chance alone. The longest gestation period ever recorded in this herd was 116 days; 6 days less than this experimental animal. All of the young were of normal size at birth and one is therefore tempted to interpret this as a possible delayed implantation. Although not a common phenomenon in pigs, some rare cases of prolonged gestation or superfetation in other animals have been interpreted as explainable by delayed implantation (BURROWS[6]).

The complete failure of pregnancy in the 2 animals receiving luteal phase protein antiserum could happen by chance, but considering that pregnancy did not fail in any of the 100 animals used to establish the colony average, it seems to be an unplausible explanation. Even if fertilization had failed with the first service, the animals would normally have recycled in 21–30 days. One of these animals recycled after 173 days and the other one had not come into estrus again at the time of this writing which was 237 days. The swineherd at Colorado State University, a man of extensive experience, claims that these 2 animals showed typical signs of early pregnancy in that they had 'bellied down' (shown enlargement of abdominal region) about 3 weeks after breeding and then regressed slowly thereafter. Assuming the accuracy of this observation, one can only conclude that a resorption must have occurred at a time coincident with early post-implantation.

The failure of these animals to return to estrus after early termination of pregnancy leads one to suspect that they were retaining a progesterone domination. Hysterectomy of gilts after estrus has been shown to cause retention of the corpora lutea with subsequent production of progesterone for prolonged periods; typically 120–200 days elapse before the animals return to estrus, an observation commonly interpreted as a block to normal luteolysis[7,8]. Dr. F. BAZER of the University of Florida has suggested that the rabbit antisera used on the pigs in the experiments described here may contain antibodies against a luteolytic factor. Immunological blockage of this factor might cause the retention of the corpora lutea and account for the failure to recycle. This question will be examined[9].

Zusammenfassung. Der Einfluss von heterologen Antiseren gegen Uterusantigene auf die Schwangerschaft wird am Modell des Schweines und des Kaninchens studiert. In diesem Versuchstier werden Anhaltspunkte für eine beeinträchtigende Wirkung der Antiseren gefunden.

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[6] H. BURROWS, J. Obstet. Gynaec. Br. Commonw. *61*, 762 (1954).
[7] L. L. ANDERSON, R. L. BUTCHER and R. M. MELAMPY, Endocrinology *69*, 571 (1961).
[8] H. G. SPIES, D. R. ZIMMERMAN, H. L. SELF and L. E. CASIDA, J. Anim. Sci. *19*, 101 (1960).
[9] The author is grateful to Dr. DONALD WILL for providing the pigs used in this study and for their breeding and maintenance.

Pineal Gland Changes of Rats Exposed to Heat

Photic stimuli, i.e. continuous light and darkness, may act on the endocrine glands through the pineal gland since the removal of this organ abolishes the effect of environmental lighting on the endocrine system[1]. It has been suggested that the pineal gland might also mediate environmental influences other than light[2]. For example, exposure of rats to low temperature was found to bring about a decrease in weight of ovaries and uteri[3] and to produce hypertrophy and hyperplasia of the pineal gland[4]. The effects of cold on the female gonads, however, were not erased by pinealectomizing the animals[3]. High temperatures (34–36 °C), too, have been found to cause changes in rat endocrine function, these being reflected in weight reduction of the hormone excreting glands[5,6]. The total body weight of rats exposed to heat has also been found to be reduced[6]. No reports, however, on the effect of heat on the pineal gland have been found in the literature.

Materials and methods. Female rats of the Hebrew University 'Sabra' strain, 21 days old and weighing 36–45 g each, were divided into 2 groups: one being exposed to constant heat of 33 ± 1 °C and the other, which served as control, was kept in a room with controlled temperature of 23 ± 1 °C. The rats were housed 6 to a cage and fed ad libitum. Light was provided by overhead fluorescent tubes which were automatically switched on at 07.00 h and off at 19.00 h each day.

Effect of continous heat on pineal weight, protein, RNA and DNA contents per pineal and total body weight of rats (± S.D.)

	Days of exposure					
	10		20		30	
Temperature (°C)	23 ± 1	33 ± 1	23 ± 1	33 ± 1	23 ± 1	33 ± 1
Pineal weight (mg)	—	—	1.13 ± 0.26	1.04 ± 0.28	1.13 ± 0.10	1.07 ± 0.14
Protein (μg)	82.8 ± 6.4	82.5 ± 6.7	96.4 ± 11.6	91.7 ± 9.7	106.5 ± 13.2	93.1 ± 8.9[b]
RNA (μg)	7.58 ± 1.3	7.76 ± 1.4	9.49 ± 1.3	8.18 ± 1.3[c]	9.82 ± 1.1	7.76 ± 1.3[c]
DNA (μg)	5.47 ± 0.7	5.86 ± 0.7	6.08 ± 0.8	5.84 ± 1.2	6.14 ± 0.7	6.38 ± 0.8
Total body weight (g)	70.4 ± 8.2	65.5 ± 6.9[a]	113.9 ± 7.4	99.9 ± 8.1[c]	147.1 ± 7.2	128.2 ± 8.1[c]
Number of samples	22	23	18	18	11	12

Each sample was pooled from 6 rats. [a]$p<0.05$, [b]$p<0.02$; [c]$p<0.01$.

After 10, 20 and 30 days' exposure to high temperature (i.e. on day of life 31, 41 and 51) rats were sacrificed by neck fracture, weighed and their pineal glands removed, rinsed in cold saline to remove blood and frozen by liquid air. 6 frozen pineals from rats in each cage were pooled in a glass tube and homogenized under cooling in 0.01 *M* NaCl. From this sample RNA, DNA and protein were determined according to the procedure outlined in an earlier publication[7].

Results. From the Table it can be seen that exposure of rats to increased environmental temperature (33 ± 1 °C) for 10 days caused no change in the pineal RNA, DNA and protein levels when compared to those of animals kept at 23 °C. Following 20 days' exposure RNA content of the gland was reduced and after 30 days pineal protein was significantly diminished, too. There was no change in the weight of the pineal gland in any of the groups tested when compared with that of their controls. Neither was there any change in pineal DNA content.

Total body weight was decreased after 10 days' exposure to heat and remained so during the entire observation period of 30 days (Table). In consequence, when comparing pineal RNA and protein with total body weight, no difference becomes noticeable between the changes due to elevated temperatures and the control values. Moreover, the unchanged pineal weight and DNA levels in heat exposed animals appear elevated when compared to body weights.

Discussion. Continuous exposure of maturing female rats to elevated temperature of 32–34 °C causes first a decrease in RNA (after 20 days) and later also in protein contents of the pineal (after 30 days). A similar effect of a decrease in pineal metabolic activity was observed in rats exposed to continuous light[7,8]. 10 days' exposure to heat, however, caused no apparent change in the levels of RNA and protein, whereas such changes were already evident after 10 days' exposure to constant light. Moreover, a simultaneous decrease in total body weight occurred at 33 °C, which may indicate that the diminished pineal RNA and protein result from a general inhibition of metabolism, but on the other hand, no decrease in pineal weight occurred. There appears also to be a correlation between the changes described and food intake, since the rats kept at 33 °C consumed 12–14% less than their controls maintained at 23 °C.

Our findings of decreased pineal metabolism in rats exposed to heat correspond with those of MILINE et al.[4] who observed hypertrophy and increased metabolic activity of the pineal in adult rats kept at low temperatures (3–10 °C).

The fact that pineal DNA content is unchanged in rats exposed to heat indicates that high temperature does not interfere with the number and multiplication of pineal cells. The similar changes in pineal protein and RNA content and time of their appearance observed with continuous heat and constant light suggest that heat stimuli, like those of light, may inhibit pineal sympathetic tone by curbing noradrenaline release.

There is general agreement that the pineal gland exerts an inhibitory effect on gonadal function[1]. The occurrence of decreased pineal metabolism through exposure to elevated temperatures which induce accelerated vaginal opening and an increase in number of corpora lutea per rat[9], is well in accord with this consensus.

On the basis of the foregoing findings, it may be postulated that the pineal gland in rats could also be transducing temperature stimuli in addition to those of light.

Zusammenfassung. Ratten, die 20 oder mehr Tage bei 33 °C gehalten werden, weisen — absolut gemessen — erniedrigten RNS- und Gesamtprotein-Gehalt der Pineals auf, während DNS und Drüsengewicht konstant bleiben. Da die Tiere durch die Hitzeexplosion an Gewicht verlieren, wird der RNS- und Proteingehalt-Abfall pro Körpergewicht nicht wahrnehmbar.

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[1] R. J. WURTMAN, J. AXELROD and D. E. KELLY, *The Pineal* (Academic Press, New York, London 1968).
[2] W. B. QUAY, Physiol. Behav. *5*, 353 (1970).
[3] R. J. REITER, J. Reprod. Fert. *16*, 217 (1968).
[4] R. MILINE, V. DEVEČERSKI, N. ŠIJAČKI and R. KRSTIĆ, Hormones *1*, 321 (1970).
[5] L. P. HERRINGTON and J. H. NELBACH, Endocrinology *30*, 375 (1942).
[6] D. E. RAY, C. B. ROUBICEK and M. HAMIDI, Growth *32*, 1 (1968).
[7] I. NIR, N. HIRSCHMANN, J. MISHKINSKY and F. G. SULMAN, Life Sci. *8*, II, 279 (1969).
[8] I. NIR, N. HIRSCHMANN and F. G. SULMAN, Proc. Soc. exp. Biol. Med. *133*, 452 (1970).
[9] S. DIKSTEIN, Y. KAPLANSKI, Y. KOCH and F. G. SULMAN, Life Sci. *9*, I, 1191 (1970).
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