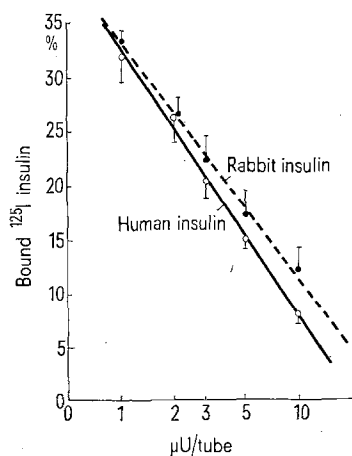


and BERSON³). This communication reports on the relative cross-reactivities of human and rabbit insulins with antibodies to porcine insulin developed in guinea-pigs.

Methods. Concentration-reactivity curves for crystalline human and rabbit insulin were determined using a modification of the double-antibody, radioimmunoassay technique of HALES and RANDLE⁴. The reagents for these determinations and human insulin were obtained from Schwarz-Mann, Orangeburg, N.Y. Single component rabbit insulin, lot No. 615-1079B-72, was prepared and kindly supplied by Dr. MARY A. ROOT of Eli Lilly and Company. After filtration, the complexed radioactive insulin was counted in a Nuclear-Chicago Mark I liquid scintillation system. Curves for both types of insulin were determined simultaneously on 6 separate occasions. The results were analyzed statistically for linearity of regression and for significance of difference between regression coefficients (BATSON⁵).



Cross-reactivity of unlabeled human insulin and unlabeled rabbit insulin versus ^{125}I -labeled pork insulin with guinea-pig antiporcine antibodies. The guinea-pig antibodies to porcine insulin do not distinguish between human and rabbit insulin. Each point on the curve is the mean value of at least 6 experiments. Bars on each point represent the standard errors.

Results and discussion. The concentrations of human and rabbit insulins that were compared ranged from 1–10 $\mu\text{U}/\text{tube}$. As shown in the Figure, this range of concentrations covers that portion of the insulin reactivity curves that can be best described as linear. Furthermore, the difference in the calculated regression coefficients were not statistically significant. The greatest difference between individual points on the curves was at the highest concentration tested, 10 $\mu\text{U}/\text{tube}$.

These data demonstrate that at the concentration tested human insulin and rabbit insulin cross-react with similar affinity to guinea-pig antibodies to porcine insulin. Rabbit insulin has been shown to cross-react with antisera to both porcine and bovine insulins to the same extent as does porcine insulin (personal communications from Dr. MARY A. ROOT). Since human, bovine, porcine and rabbit insulins differ only in the C-terminal amino acid of the B chain, this suggested that the C-terminal amino acid is not a critical antigenic site with regard to the binding of the above insulins to guinea-pig antiporcine insulin antibodies.

Résumé. L'insuline du lapin diffère de l'insuline de l'homme et du porc par l'acide aminé C-terminal de la B-chaîne. Les expériences démontrent que les insulines humaine et cuniculine réagissent de la même manière envers l'anticorps du cobaye et l'insuline du porc. Par conséquent, l'acide aminé en question ne paraît pas être déterminant pour la production des anticorps contre les insulines de l'homme et du lapin.

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Pituitary Sialic Acid Concentration During the Estrous Cycle of Rats

Cyclic changes in the concentration of follicle-stimulating hormone (FSH) have been observed in the pituitary glands of female rats during estrous cycle^{1–4}. Purified FSH and luteinizing hormone (LH) of ovine and human origin contain sialic acid⁵. Analysis of ovine and human purified gonadotrophins showed that FSH has a much higher content of sialic acid than does LH^{6–8}, and release of sialic acid from the FSH preparation by incubation with neuraminidase results in an almost total loss of biological activity of the hormone^{9,10}. RENNELS and HOOD¹¹ suggested that the increased concentration of pituitary sialic acid following ovariectomy in rats is due to the increasing levels of FSH. Recently WARD et al.¹² reported the absence of sialic acid in rat LH. In view of these facts, it was of interest to see whether variations could be found in the concentration of pituitary sialic acid of female rats during the estrous cycle.

Colony bred, 3-month-old female rats of Holtzman strain were used. Vaginal smears of 60 rats were taken

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³ J. A. MCCLINTOCK and N. B. SCHWARTZ, *Endocrinology* **83**, 433 (1968).

⁴ A. NEGRO-VILAR, M. SAR and J. MEITES, *Endocrinology* **87**, 1091 (1970).

⁵ U. GROSCHER and C. H. LI, *Biochim. biophys. Acta* **37**, 375 (1960).

⁶ E. F. WALBORG JR. and D. N. WARD, *Biochim. biophys. Acta* **78**, 304 (1963).

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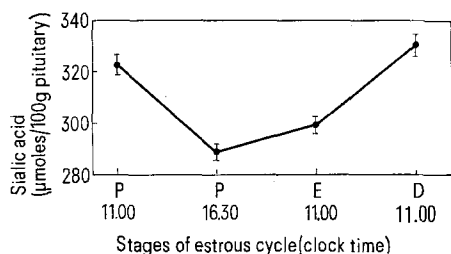
¹⁰ K. F. MORI, *Endocrinology* **85**, 330 (1969).

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each day between 10.00 and 11.00 h and again when necessary at 16.30 h. Only those rats which exhibited at least 2 consecutive 4-day cycles were used. 10 rats in each group were sacrificed by decapitation on the days of proestrus, estrus, and diestrus (but not metestrus) at 11.00 h or 16.30 h. The pituitary glands were dissected out quickly, posterior lobe was discarded and the anterior lobe weighed individually to the nearest 0.2 mg on a torsion balance, homogenized in cold 0.1 N H₂SO₄, hydrolyzed at 80°C for 1 h and the sialic acid was estimated by the thiobarbituric acid method of WARREN¹³. The *p*-values were calculated using student's *t*-test.

The concentration of sialic acid in the pituitary gland during different phases of estrous cycle is shown in the Figure. The highest concentration of sialic acid in the pituitary was seen on the morning of the day of proestrus. By 16.30 h on the same day, the concentration decreased significantly (*p* < 0.01) to the lowest level observed during the cycle. The sialic acid level remained low at estrus to the value close to that observed during the afternoon of proestrus. Thereafter, a marked increase in the sialic acid concentration was evident by 11.00 h on the day of diestrus.



Changes in the pituitary sialic acid concentration during the estrous cycle of rats. P, proestrus; E, estrus; D, diestrus. Each point represents 10 pituitaries.

Variations in the concentration of pituitary sialic acid observed are similar to the fluctuations in the pituitary FSH concentration in female rats during different phases of estrous cycle. CALIGARIS et al.¹ and NEGRO-VILAR et al.⁴ observed a sharp decline in pituitary FSH concentration between 09.00 h and 17.00 h on the afternoon of proestrus. Also a drop in the pituitary FSH and LH levels is seen between proestrus and estrus, reflecting the discharge of an ovulation-inducing surge of gonadotrophins on the afternoon of proestrus^{3,14,15}. Thereafter, FSH levels increased until the next proestrus. Since sialic acid is absent in rat LH¹², the changes in the pituitary sialic acid concentration of the two sexes are more closely correlated with FSH levels than LH. Thus the close correlation in the changing levels of pituitary FSH and sialic acid during different phases of estrous cycle suggests that sialic acid levels in the pituitary gland may be taken as an indicator of FSH content.

Zusammenfassung. Bei der Ratte wurde der Sialinsäuregehalt des Hypophysenvorderlappens während des Prooestrus, Oestrus und Dioestrus bestimmt. Da das Luteinhormon der Ratten keine Sialinsäure enthält, können die Schwankungen des Sialinsäuregehaltes als Mass für die FSH-Konzentrationen genommen werden.

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Termination of Diapause by Juvenoids in Two Species of Ladybirds (Coccinellidae)

The juvenoids or juvenile hormone analogues are known to initiate egg development in diapausing adult insects^{1,2}. For example, methyl 10,11-epoxyfarnesoate induces reproduction in beetles *Hypera postica*³ and *Oulema melanopus*⁴, and methyl farnesoate was reported to stimulate previtellogenesis in *Pterostichus nigrata*⁵. The present study compares activities of 18 juvenoids⁶ and describes their effects on diapausing adults of beetles *Semiadalia undecimnotata* (Schneider) and *Coccinella septempunctata* Linnaeus (Coccinellidae).

The life-cycle of aphidophagous coccinellids includes a long period of diapause that occurs usually in the adult stage. In the thermo- and xerophilous *S. undecimnotata*, the main distribution area of which lies in Southern Europe and Asia the diapause lasts from late July or early August to late April or May. The beetles migrate to permanent hibernation quarters where they form large aggregations. Although in the open the beetles remain inactive in spite of the high temperatures of late summer and early autumn, they can easily be activated under long-day conditions (18 h photophase) and high temperature (e.g. 20–23°C) when provided with appropriate food, i.e. certain aphids⁷. To prove that activation was initiated by the application of juvenoids, the diapause promoting photoperiod of 12 h light and 12 h dark was used in the present experiments.

In *C. septempunctata*, which is the most common coccinellid in Central Europe, most of the beetles diapause in small groups for 7–8 months. The diapause is rather stable. Exposure of beetles to a long photophase before October activates only 15–20% of diapausing specimens. In the course of diapause development in subsequent months the endogenous inhibition of reproduction gradually ceases and the beetles start to respond more

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⁶ The juvenoids I, II, VI, VII, VIII, XII, XIII, XIV, and XVIII were kindly provided by Drs. M. ROMAŇUK, V. JAROLÍM, P. BERAN, Z. ARNOLD and Prof. F. ŠORM of the Institute of Organic chemistry and Biochemistry, ČSAV, Prague; the remaining juvenoids were obtained through the courtesy of Dr. J. B. SIDDALL of the Zoecon Corporation, Palo Alto, California. All juvenoids were mixtures of isomers; the aliphatic compounds contained about 80% of the 2-*trans* isomers.

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