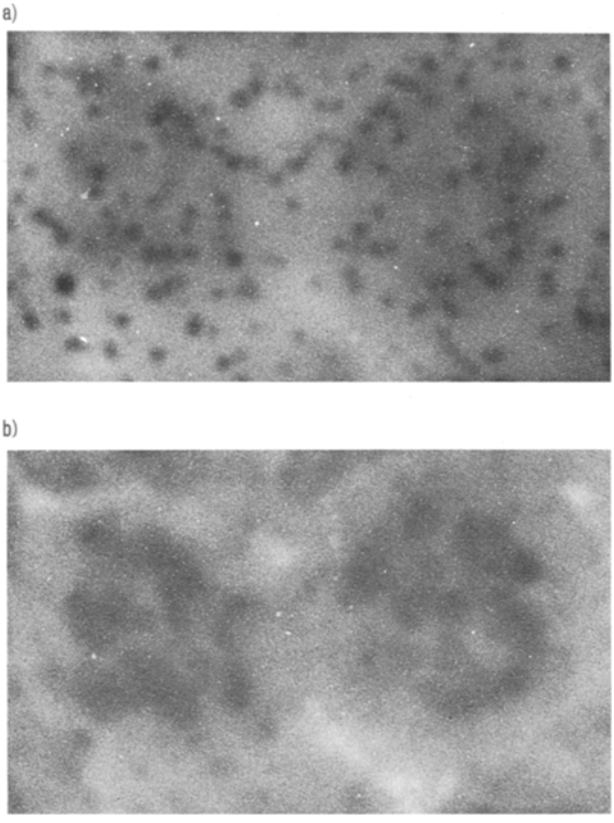

Fig. 1. Microautoradiogram of 2 dividing ovarial follicular granulosa cells from a mouse 4 h after a s.c. injection of ^{3}H-estradiol. In a) the grains of the emulsion are focussed, whereas in b) the toluidine blue stained section is in focus. ×3200.

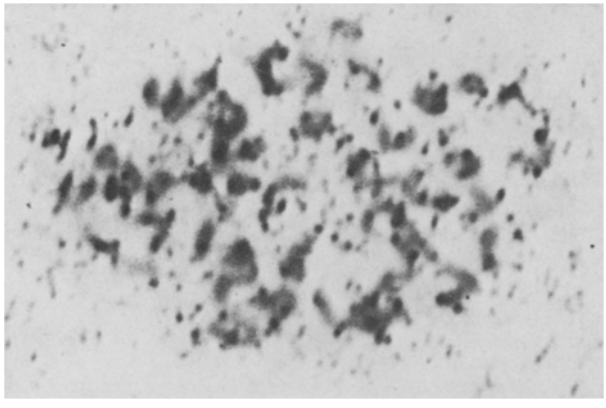

Fig. 2. Microautoradiogram of chromosomes in an ovarial squash preparation from a mouse 4 h after a s.c. injection of ^{3}H-estradiol. An accumulation of silver grains can be seen over the chromosomes. ×2800.

pieces. For this purpose 12 mice were treated as described for the mice used for autoradiography except that they only received 5 μCi of ^{3}H-estradiol. In 6 mice the ovaries were then treated according to the squash preparation method and in the other 6 mice the ovaries were treated for Epon-embedding. The loss of radioactivity in each of the different solutions used during the procedures was then determined in a Packard liquid scintillation counter. It was found that during the squash preparation 41.6 ± 10.4% (mean ± S.D.) of the radioactivity was lost from the treated pieces. During the Epon-embedding 22.1 ± 8.0% (mean ± S.D.) was lost.

The autoradiograms obtained from the Epon-embedded sections showed that there was an accumulation of silver grains over chromosomes of the frequently dividing granulosa cells (Figure 1). The accumulation was more pronounced in some cells than in others. In non-mitotic granulosa cells a nuclear accumulation of radioactivity could also be seen. In the squash-preparations a localized accumulation of radioactivity could be observed over chromosomes (Figure 2). Also in this case there was some variation in the amount of labelling between different mitotic figures. Silver grains over chromosomes from control mice were not observed.

The results of the present investigation constitute visual evidence that the nuclear binding of estradiol in target cells represent a binding to the chromosomal/chromatin part of the nucleus. There was, as mentioned, a loss of radioactivity during the various steps of tissue-preparation and it therefore cannot be excluded from the present results that in addition a nonchromosomal nuclear steroid-binding site exists. The chromosomal binding must, however, in such a case be the most stable one. Both the acidic proteins and the basic histones of the chromatin, but not DNA, have been reported to possess steroid-binding properties[10,15,16]. The acidic proteins seem, however, to be the most important candidates as site of action of estrogen at the genomic level[17].

Zusammenfassung. ^{3}H-Oestradiol wurde Mäusen injiziert und nachher autoradiographisch verfolgt, wobei Ag-Körnchen über den Chromosomen der Granulosazellen des Ovariums festgestellt werden konnten.

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12 March 1973.

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Rabbit and Human Insulins: Similar Cross-Reactivities with Antibodies to Porcine Insulin

Rabbit insulin differs from human and porcine insulins in the C-terminal amino acid of the B chain (SMITH[1]). It has been suggested that the C-terminal of the B chain is a potential site of antigenicity of bovine and porcine insulins in man (BERSON and YALOW[2]). Although species differences in insulin have been discerned, guinea-pig antiporcine insulin serums generally discriminate weakly or not at all between human and porcine insulins (YALOW

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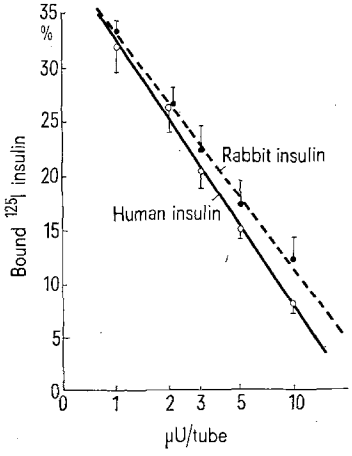
and BERSON[3]). This communication reports on the relative cross-reactivities of human and rabbit insulins with antibodies to porcine insulin developed in guinea-pigs.

Methods. Concentration-reactivity curves for crystalline human and rabbit insulin were determined using a modification of the double-antibody, radioimmunoassay technique of HALES and RANDLE[4]. 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 (BATSON[5]).



Cross-reactivity of unlabeled human insulin and unlabeled rabbit insulin versus I^{125}-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 μU/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 μU/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 antiserums 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 quéstion 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 acid[5]. 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 HOOD[11] 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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