number of other species (FRERICHS and CREUTZFELDT[10]). Amelioration of the diabetic state or of the insulin resistance, like that reported by SOLOMON and MAYER[11] and MAHLER and SZABO[12], was not observed.

The obvious discrepances between our results and those of SOLOMON and MAYER[11] may best be explained by the differences between the two investigations with regard to the route of drug administration and the alloxan doses employed. The former authors gave alloxan intraperitoneally as compared to the intravenous injections given in the present study. Also, the doses used by SOLOMON and MAYER[11] were based on lean body mass, which means that the amount of alloxan given in their study was about half that presently used. Against this background it is concluded that alloxan is diabetogenic in obese-hyperglycemic mice and that it acts by a mechanism similar to that in other species. Since B-cells constitute more than 90% of the islets of obese-hyperglycemic mice, isolated intact islets of such animals should be an excellent preparation for further studies of the mechanism for the selective destruction of B-cells by alloxan (HELLERSTRÖM and GUNNARSSON[13]).

Zusammenfassung. Nachweis, dass fettsüchtige, hyperglykämische Mäuse nach Alloxan-Injektion einen erhöhten Blutzuckerspiegel und herabgesetzte Insulinkonzentrationen im Vergleich mit den unbehandelten Kontrolltieren zeigen. B-Zellen der Langerhansschen Inseln zeigen nach Alloxan nekrotische Veränderungen.

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27 April 1973.

[10] H. FRERICHS and W. CREUTZFELDT, in *Handbuch des Diabetes Mellitus, Vol. I.* (Ed. E. PFEIFFER; J. F. Lehmanns Verlag, München 1969), p. 811.
[11] J. SOLOMON and J. MAYER, Nature, Lond. *193*, 135 (1962).
[12] R. J. MAHLER and O. SZABO, Am. J. Physiol. *221*, 980 (1971).
[13] C. HELLERSTRÖM and R. GUNNARSSON, Acta diabetol. lat. *7*, 127 (1970).

Chromosomal Accumulation of ^{3}H-Estradiol in Dividing Ovarial Granulosa Cells and Ovarial Squash Preparations

The biological mechanism of action of many steroid hormones has been attributed to an interaction with genetic information in target cell nuclei[1–4]. Steroid hormones can, via such a mechanism, exert an important influence on the transport from the nucleus and accumulation in the cytoplasm of RNA and ribonucleoprotein particles[5].

In target cells most steroids are combined with a specific protein receptor in the cytoplasm and transported into the cell nuclei[6,7]. In the nuclei such steroids can be found associated with chromatin[8,9]. A binding to non-chromosomal proteins may, however, also exist[10].

The present investigation has been performed to see if it is possible by autoradiographical methods to demonstrate an accumulation of a steroid in the chromosomes of a dividing cell of a target tissue. Estradiol was chosen as the steroid and the granulosa cell layer of the ovary was chosen as the target tissue. Estradiol has been shown to be strongly accumulated in the nuclei of the granulosa cell layer of the follicle, which supports the idea that it is a target for estradiol[11,12]. This tissue was chosen because the number of mitoses is easily stimulated and because the cells are large and therefore suitable to study under the microscope.

In 2 female albino NMRI-mice, weighing 20 g each, the follicle growth was stimulated by a s.c. injection of 50 IU pregnant mare's serum (PMS, obtained from AB Ferring, Sweden); 24 h later this treatment was repeated. The mice were at this time also injected s.c. with 1 mCi ^{3}H-estradiol (2,4,6,7-^{3}H-estradiol-17β, spec. act. 110 Ci/m*M*, New England Nuclear; dose per animal: 2,5 μg) dissolved in 0.1 ml dimethyl sulfoxide (DMSO). 1 h later, the mice were injected i.v. with 2.5 mg/kg colchicine, (Sigma Chemical Co., USA), which arrests the mitotic division in metaphase. The mice were killed 3 h after this injection. One ovary in each mouse was taken and cut into 5 pieces and fixed in Levans fixative (60 ml glacial acetic acid, 10 ml 1 *N* hydrochloric acid and 30 ml distilled water) for 20 min. The pieces were then stained with a few drops of orcein (2 g orcein, 60 ml glacial acetic acid and 40 ml distilled water). Thereafter each piece was placed between 2 slides and squashed. The squash-preparations were then subjected to conventional stripping film autoradiography[13] by which stripping film (Kodak AR 10) is stretched on water and floated on to the preparations. Pieces of ovaries from control mice, not injected with ^{3}H-estradiol, were also taken and treated as described above. The other 2 ovaries from the ^{3}H-estradiol-injected mice were cut into small pieces and put in a cold isotonic solution of 1% osmiumtetroxide buffered to pH 7.4 and fixed for 2 h. After dehydration in ethanol the tissue pieces were embedded in Epon. Sections, 1 μm thick, were cut on an LKB Ultrotome and put on glass-slides. Autoradiography was then performed as described for the squash-preparations. The autoradiograms were exposed at −15 °C, the exposure-time being 3–6 months. Since steroid hormones are known to be diffusible during the various steps of the histologic and autoradiographic processing[14], control experiments were undertaken to estimate the loss of radioactivity in the

[1] P. KARLSSON, Dent. med. J. *86*, 668 (1961).
[2] J. S. EDELMAN, R. BOGORACH, G. A. PORTER, Proc. natn. Sci. USA *50*, 1169 (1963).
[3] F. T. KENNEY and W. L. ALBRITTON, Proc. natn. Acad. Sci. USA *45*, 1693 (1965).
[4] T. H. HAMILTON, C. C. WIDNELL, J. R. TATA, J. biol. Chem. *243*, 408 (1968).
[5] J. R. TATA, Nature, Lond. *219*, 331 (1968).
[6] D. TOFT and J. GORSKI, Proc. natn. Acad. Sci. USA *55*, 1574 (1966).
[7] E. V. JENSEN, T. SUZUKI, T. KAWASHIMA, W. E. STUMPF, P. W. JUNGBLUT, E. R. DESOMBRE, Proc. natn. Acad. Sci. USA *59*, 632 (1968).
[8] R. B. J. KING and D. R. INMAN, J. Endocr. *35*, 26 (1966).
[9] A. W. STEGGLES, T. C. SPELSBERG, S. R. GLASSER, B. W. O'MALLEY, Proc. natn. Acad. Sci. USA *68*, 1479 (1971).
[10] M. SLUYSER, in *The Biochemistry of Steroid Hormone Action* (Ed. R. M. S. SMELLIE; Academic Press, London/New York 1971), p. 31.
[11] S. ULLBERG and G. BENGTSSON, Acta endocr. Copenh. *43*, 75 (1963).
[12] W. E. STUMPF, Endocrinology *85*, 31 (1969).
[13] J. DONIACH and S. P. PELC, Br. J. Radiol. *23*, 184 (1950).
[14] W. E. STUMPF, Am. Zoologist *11*, 725 (1971).

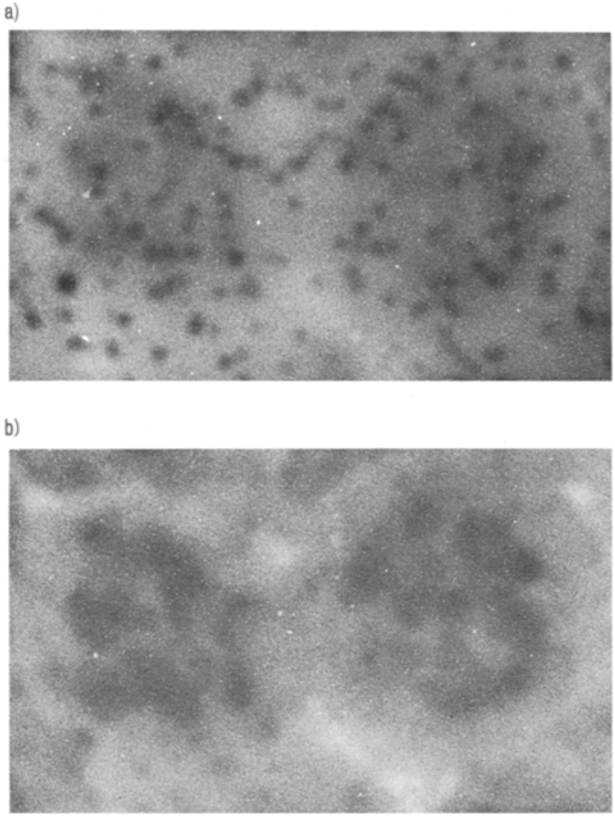

Fig. 1. Microautoradiogram of 2 dividing ovarial follicular granulosa cells from a mouse 4 h after a s.c. injection of ^{3}H-estradiol. In a) the grains of the emulsion are focussed, whereas in b) the toluidine blue stained section is in focus. ×3200.

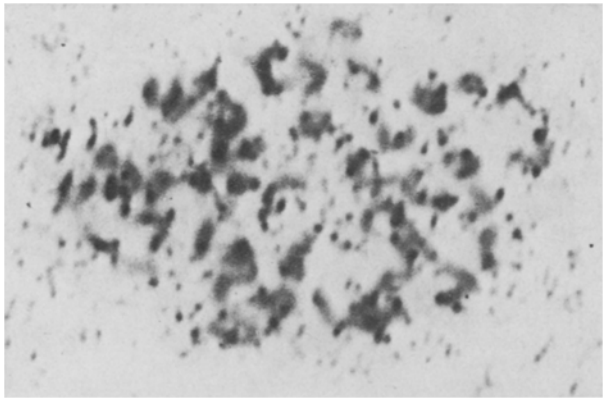

Fig. 2. Microautoradiogram of chromosomes in an ovarial squash preparation from a mouse 4 h after a s.c. injection of ^{3}H-estradiol. An accumulation of silver grains can be seen over the chromosomes. ×2800.

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.

[15] R. J. B. KING and J. GORDON, J. Endocr. *40*, 195 (1968).
[16] R. J. B. KING, J. GORDON, A. W. STEGGLES, Biochem. J. *114*, 649 (1969).
[17] T. H. HAMILTON, in *The Biochemistry of Steroid Hormone Action* (Ed. R. M. S. SMELLIE; Academic Press, London/New York 1971), p. 49.

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

[1] L. F. SMITH, Am. J. Med. *40*, 662 (1966).
[2] S. A. BERSON and R. S. YALOW, Am. J. Med. *40*, 676 (1966).