precipitation bands when reacted with rat plasma in the double immunodiffusion test. The duct of the right parotid gland of rats was cannulated with a polyethylene catheter[6] and 0.5 ml of the antiserum to rat plasma, normal rabbit serum, normal human serum or physiological saline were slowly introduced. The rats were killed 24 h after the intubation and the right gland was removed for histological examination. The extent of inflammation was evaluated on an arbitrary scale from 0 to 3+ as described elsewhere[7]. Since proteinous solutions per se cause mild inflammation (1+), moderate (2+) and severe lesions (3+) only were considered to be of significance.

Moderate to severe sialoadenitis developed in 8 of 12 rats following instillation of antiserum to rat plasma into the parotis. Histologically, the lesions were characterized by inter- and intralobular inflammatory infiltration and focal necrosis of the parenchyma (Figures 1 and 2). The infiltrate consisted of polymorphonuclear granulocytes with an admixture of lymphocytes, histiocytes and a few plasma cells. Moderate sialoadenitis (2+) was observed in 1 of 20 control rats given normal human serum. The glands of 10 and 9 rats receiving normal rabbit serum or physiological saline, respectively, were normal.

Blood plasma proteins are widely distributed in cells, connective tissues and interstitial spaces, lymphatics and blood vessels[9]. Instillation into the gland of antiserum to plasma components is apparently followed by local formation of immune complexes, resulting in an inflammatory reaction[10]. It is assumed that the plasma proteins residing in cells, connective tissues, interstitial spaces and/or vessels participate in the formation of the complexes. In view of the procedure employed, sialoadenitis is ascribed to a modified reversed Arthus type of reaction, since it is caused by precipitating antibodies introduced into a tissue harbouring the respective soluble antigens[11,12].

Zusammenfassung. Durch den Ausführungsgang der Ohrspeicheldrüse wurde Antiserum gegen Rattenblutserum eingeleitet, was zu einer schweren allergischen Entzündung der Parotisdrüse führte. Es wird angenommen, dass es sich bei dieser Entzündung um eine besondere Art von Arthusreaktion handelt.

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[1] O. Haferkamp, Virchows Arch. Path. Anat. *335*, 298 (1962).
[2] O. Haferkamp, Verh. dt. Ges. inn. Med. *67*, 722 (1962).
[3] W. C. Chan, J. Path. Bact. *88*, 592 (1964).
[4] R. Bevilacqua and L. Mosca, Boll. Soc. ital. Biol. sper. *44*, 830 (1968).
[5] Y. Kurata, Y. Watanabe, S. Okada and Y. Fukuyama, Int. Arch. Allergy *35*, 392 (1969).
[6] M. Ulmansky, Y. Sela, T. Dishon, E. Rosenmann and Y. H. Boss, Arch. oral Biol. *17*, 609 (1971).
[7] Y. Sela, M. Ulmansky, T. Dishon, E. Rosenmann and Y. H. Boss, Virchows Arch. Path. anat., Abt. A *355*, 213 (1972).
[8] E. Rosenmann, Y. Sela, M. Ulmansky, T. Dishon and Y. H. Boss, Manuscript submitted for publication.
[9] D. Gitlin, B. H. Landing and A. Whipple, J. exp. med. *97*, 163 (1953).
[10] B. Benacerraf and R. T. McClusky, A. Rev. Microbiol. *17*, 263 (1963).
[11] J. H. Humphrey and R. G. White, *Immunology for Students of Medicine*, 3rd edn. (Blackwell Scientific Publications, Oxford and Edingburgh 1970), p. 449.
[12] This investigation was supported by a grant from Nessim David Gaon of Geneva.

7α-Hydroxylation of Dehydroepiandrosterone in Human Testis and Epididymis in vitro

Steroid 7α-hydroxylase, known from various tissue of animals and lower vertebrates, was demonstrated in human adrenals and liver[1]. The testicular 7α-hydroxylation of steroids has so far been described only in human foetus when dehydroepiandrosterone was used as a substrate[2], and in rats where androstenedione was the preferred substrate for hydroxylation[3,4].

Considering the possible regulative role of 7α-hydroxylation in the androgen biosynthesis[3], we studied the in vitro formation of 7α-hydroxylated metabolites of dehydroepiandrosterone in human testis and epididymis of an adult man.

Human testes and epididymis were obtained from a sexually deviated but otherwise healthy man aged 38 years, who underwent the voluntary castration. The histological control confirmed the normal structure of both testis and epididymis as well as the ability of the testes for complete spermiogenesis. Immediately after operation the tissues were kept in an ice bath and then minced separately for approximately 30 min. The samples of minced tissue, 100 mg each, were incubated separately with 0.2 μC of [4-^{14}C] dehydroepiandrosterone ([4-^{14}C] 3β-hydroxy-5-androsten-17-one = ^{14}C-DHA; specific activity 14.0 mCi/m*M*), dissolved in 0.05 ml propylene glycol. The incubation was carried out in 3 ml Krebs-Ringer buffer, pH 7.4, containing glucose, in an oxygen atmosphere at 37 °C for 60 min. Both tissues were incubated in 2 parallel samples without addition of any cofactor, with the blank containing only a substrate and buffer.

After the incubation, 20 μg of each the expected non-radioactive metabolite was added to the samples and the incubation mixture was extracted with ethyl acetate and chloroform. The extracts were purified by chromatography on small columns of silica gel followed by paper chromatography in the system Bush B5. The more polar metabolites (7-hydroxydehydroepiandrosterone and 5-androstene-3β, 7α, 17β-triol) were separated in this system, whilst the less polar ones were rechromatographed in the system, cyclohexane: toluene: methanol: water = 9:1:8:2. 7α- and 7β-hydroxyepimers of dehydroepiandrosterone were further separated in the system n-heptane: toluene: methanol: water = 9:11:16:4 developed for 48 h. 5-androstene-3β, 7α, 17β-triol was rechromatographed on ethylene glycol impregnated paper, developed by dichloromethane for 10 h, respectively. The radioactive steroids on the paper were detected by autoradiography, the spots were then cut off and their radioactivity was measured using liquid scintillation spectrometer. Additional 500 mg of tissue was incubated with [4-^{14}C] dehydroepiandrosterone (2.5 μCi) and, in the ex-

[1] L. Stárka, Naturwissenschaften *52*, 499 (1965).
[2] J. Šulcová, J. E. Jirásek and L. Stárka, in *The Foeto-Placental Unit.*, (Eds. A. Pecile and C. Finzi, Excerpta med., Amst.), Internat. Congr. Ser. No. *183*, 201 (1969).
[3] H. Inano, K. Tsumo and B. Tamaoki, Biochemistry *9*, 2253 (1970).
[4] H. Inano and B. Tamaoki, Biochemistry *10*, 1503 (1971).

tract of incubation mixture, the identification of individual metabolites was carried out on a broader scale on the basis of their chromatographic properties and recrystallization to a constant specific radioactivity, respectively.

The amounts of metabolites formed by 100 mg of each tissue are shown in the Table. It is evident that 7α-hydroxylase of DHA is present in both testis and epididymis and that its activity is practically identical in both cases. However, the total metabolic activity is higher in the testis, which results from higher decrease of ^{14}C-DHA. There is a very active 17α-hydroxysteroid:dehydrogenase in the testis, as demonstrated by formation of a high yield of 5-androstene-3β, 17β-diol and testosterone. Due to the activity of this enzyme, 5-androstene-3β, 7α, 17β-triol prevailed among the 7-hydroxylated metabolites of DHA in the samples incubated with the testis. In epididymis, the dehydrogenases of 3β- and 17β-hydroxysteroids are weakly active, therefore predominantly 7α-hydoxydehydroepiandrosterone was there found. The formation of epimeric 7β-hydroxy-DHA in samples incubated with both testis and minced epididymis suggests either the presence of DHA: 7β-hydroxylase or 7α- and 7β-hydroxysteroid:dehydrogenase or 7α/7β epimerase in these tissues. However, the yield of 7β-epimer was essentially lower than that of 7α-hydroxy DHA.

The total amount of 7-hydroxylated metabolites of DHA in incubation mixtures with testis is relatively high, i.e. approx. 70% of that of testosterone formed from DHA under the same conditions. This fact agrees with the results reported by INANO et al.[3]. In their experiments, 7α-hydroxyandrostenedione was often obtained in higher yield from androstenedione than testosterone itself by microsomal fraction of mature rat testes supplemented with NADPH. They postulated a physiological role of the hydroxylated metabolite in relation to testicular endocrine function and/or to regulation of androgen production and its secretion. However, a definite comparison of our results with those of INANO et al. is difficult with respect to a different substrate, origin of the tissue and incubation conditions (cofactors). In our experiments, when we incubated both [4-^{14}C]androstenedione and [4-^{14}C]testosterone with minced human testis under the same conditions as we did with ^{14}C-DHA, 1 compound in the fraction of polar metabolites of testosterone and 2 compounds among polar metabolites of androstenedione, respectively, were found, the chromatographic mobilities of which corresponded to 7α-hydroxytestosterone and 7α-hydroxyandrostenedione. Owing to their very small yield, they could not be identified further. Unlike the rat testis, in the human testis dehydroepiandrosterone is 7-hydroxylated preferentially to androstenedione and testosterone. The finding of 7-hydroxylase activity in both testis and epididymis confirms, however, the non-specifity of this enzymatic system already known before[2,5]. The problem of physiological importance of 7-hydroxylation process in human testes remains still unexplained.

Formation of [4-^{14}C] dehydroepiandrosterone metabolites in healthy human testis and epididymis in vitro

Metabolite	Testis (dpm)	Epididymis (dpm)
DHA recovered	118,535	371,345
5-Androstene-3β, 17β-diol	108,768	984
Testosterone	15,506	315
4-Androstene-3, 17-dione	1,393	28
5-Androstene-3β, 7α, 17β-triol	9,672	469
7α-Hydroxy-DHA	826	10,747
7β-Hydroxy-DHA	238	1,223
Total of 7-hydroxylated metabolites	10,736	12,439

In each experiment 100 mg of tissue was incubated with 0.2 μCi of [4-^{14}C] dehydroepiandrosterone. The values are averages of 2 parallel experiments. The values are corrected in respect to the blank, but not to methodological losses.

Zusammenfassung. Zerhackte menschliche Hoden und Epididymis wurden mit Dehydroepiandrosteron ohne Kofaktoren bebrütet und es wurden unter anderem 7α- und 7β-Hydroxydehydroepiandrosteron und 5-Androsten-3β, 7α, 17β-triol charakterisiert. Die Ausbeute an 7-hydroxylierten Metaboliten war fast gleich in Hoden und Epididymis.

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[5] J. ŠULCOVÁ and L. STÁRKA, Experientia *19*, 632 (1963).

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Degenerating Pituicytes in the Neural Lobe of Osmotically Stressed Rats

It is well known that the pituicyte is a specialized glia-like interstitial cell interposed among the neurosecretory endings of the hypophysial neural lobe. The importance of this cell type is due to the fact that to pituicytes has been attributed, beyond a supporting role common to other glial cell types, also an intermediary role in transport and release of neurosecretory hormones[1]. In this context, the present note deals with some degenerative aspects of pituicytes in the neural lobe of dehydrated rats.

Materials and methods. 20 male adult rats of a Wistar strain were deprived of water during 5, 10, 15 days. Some rats were fed with standard diet and water ad libitum and used as controls. The samples were prefixed with 2% glutaraldehyde in phosphate or in cacodylate buffer, postfixed in osmium tetroxide, dehydrated in ethanol and embedded in Epon.

Results and discussion. Our results concern the latest stages of the dehydrating experiment, i.e. the fine pituicytic modifications appearing 10–15 days after the beginning of the experiment, being the early stages already described[2].

The lipid droplets undergo a progressive vacuolization and reduction in number. The cisternae of the smooth endoplasmic reticulum swell progressively up to assume a vacuolized and variably-shaped appearance. It is also possible to observe swollen mitochondria, disorganized Golgi complexes and some scattered lipofuscin bodies (Figure 1). Many glycogen-like particles permeate especially the peripheral areas of the pituicytic cytoplasm