Results. The activity of adenosine triphosphatase was present in the ependymal layer in both animals investigated. In the hypendyma no reaction except that in the hypendymal rosettes and ducts in the cow was revealed. A particularly strong activity was seen in the walls of the capillaries. In the posterior commissure, the reaction was also very strong. The location of the activity of adenosine triphosphatase in the S.C.O. is shown in Figure 2. In the epiphysis, a moderate activity was observed in pineal cells.

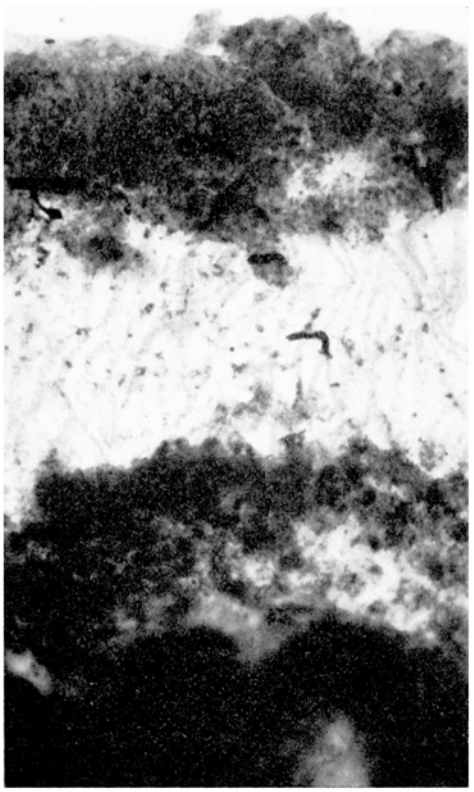

Fig. 2. Frozen section from the subcommissural organ of the cow stained for adenosine triphosphatase activity. Reaction observable in the outer ependymal layer, in the hypendymal rosettes and in the walls of the capillaries. Strong reaction in the posterior commissure. The reticular hypendymal tissue shows no reaction. PADYKULA and HERMAN's method without counterstain. 260 ×.

Discussion. The location of adenosine triphosphatase in the S.C.O. demonstrated in the present study was the same as that of a marked activity of alkaline phosphatase reported previously[4]. Alkaline phosphatase can also dephosphorylate adenosine triphosphate. It has been found out, however, that BAL at about $5 \times 10^{-3} M$ inhibits activity of alkaline phosphatase while it enhance activity of adenosine triphosphatase[3]. By using this inhibitor, no decrease was seen in activity of adenosine triphosphatase.

Generally, the adenosine triphosphatase is responsible for the breakdown of adenosine triphosphate to adenosine diphosphate with the release of a large amount of free energy for cellular activity. The physiological role of adenosine phosphatase in the S.C.O. cannot be defined in detail on the basis of present knowledge. The activity of adenosine triphosphatase, as well as the activity of the other histochemically demonstrable enzymes in the S.C.O., may indicate the high metabolic rate taking place in this organ. It can be concerned with the synthesis of the secretion which has been considered a protein[4].

Zusammenfassung. Die Aktivität der Adenosintriphosphatase im Subkommissuralorgan wurde mit der histochemischen Methode von PADYKULA und HERMANN[3] untersucht. Die Enzymaktivität konnte bei der Ratte nur in den Ependymzellen und in den Wänden der Kapillaren beobachtet werden. Bei der Kuh zeigten auch die hypendymalen Rosetten und Gänge eine deutliche Aktivität. Die vorhandene Aktivität der Adenosintriphosphatase ist wahrscheinlich ein Ausdruck des grossen Energieumsatzes in den «sekretorischen» Zellen des Subkommissuralorgans.

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Department of Anatomy and Embryology, College of Veterinary Medicine, Helsinki (Finland), November 7, 1962.

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Quantitative Analyses of Sulfur in Isolated Pancreatic Islets of Mice[1]

In the insulin molecule, two disulfide bridges link the A and B peptide chains, and a third disulfide bridge extends between cystine residues within the A chain[2]. Histochemical investigations indicate an especially high concentration of disulfide groups in the cytoplasm of the pancreatic islet B cells[3], which have recently been shown to contain no less than about 15% of their dry weight as insulin[4,5]. In view of this, it may be assumed that the total amount of sulfur in the B cells might be closely related to their insulin content. In the present investigation, the sulfur content of isolated islet tissue was studied during different conditions of insulin storage. Mice with the obese-hyperglycemic syndrome were chosen as experimental animals, since their hyperplastic islets are composed of a relatively pure B cell population[6,7]. For comparative purposes sulfur analyses were carried out also on exocrine parenchyma from both normal and obese-hyperglycemic mice.

Methods. Female mice with the manifest obese-hyperglycemic syndrome weighing about 50 g (= AO-mice) and

[1] Supported by research grant A-5759 from the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service. Our sincere thanks are also due to W. J. KIRSTEN, M.D. Institute of Medical Chemistry, University of Uppsala, for carrying out the sulphur analyses.

[2] F. SANGER, in *Currents in Biochemical Research* (D. E. Green Ed., New York 1956), p. 434.

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lean litter mates with a body weight of about 25 g (= AN-mice) were used. The animals belonged to a strain with a recessive trait for obesity and hyperglycemia originating at the Roscoe B. Jackson Memorial Laboratory, Bar Harbor (Maine, U.S.A.). Mouse bread (caloric composition: 54% carbohydrate, 16% fat and 30% protein) was supplied *ad libitum* until about 48 h before death, when food was withdrawn from 9 of the obese-hyperglycemic animals (= AOS-mice).

After killing by hyperextension of the neck, the pancreas was immediately excised and placed in a Petri dish containing ice-cold 0.25 *M* sucrose solution. Pancreatic islets were identified and dissected free from surrounding exocrine tissue under a stereo microscope (Figure). Small specimens were removed from especially thin parts of the exocrine parenchyma obviously devoid of islet tissue. Isolated pancreatic islets or samples of acinar tissue were placed on small pieces of platinum foil, which had previously been weighed on a balance (Mettler UM 7) with a sensitivity of 0.1 μg. The tissue was air dried over night at room temperature and its dry weight obtained by reweighing the foil. From each animal 3–10 islets, corresponding to a dry tissue weight of about 50–200 μg, were pooled on a foil. The weight of the exocrine tissue samples lay within the same range.

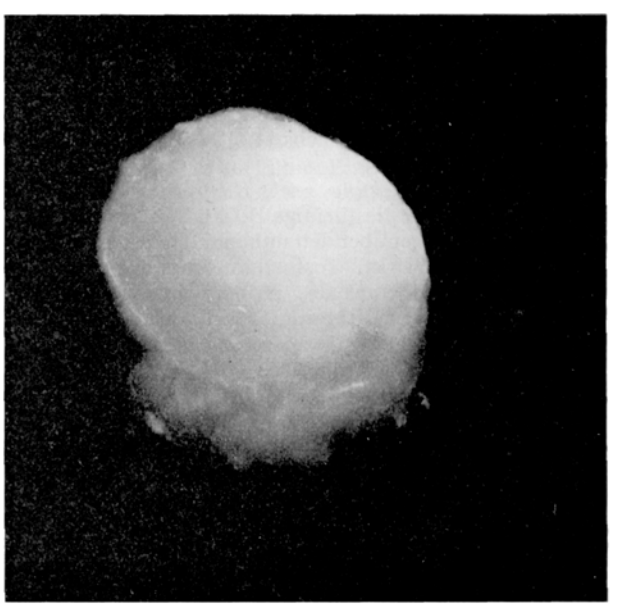
The sulfur analyses were made according to KIRSTEN[8] by dry combustion using vanadium pentoxide and subsequent hydrogenation. After converting the hydrogen sulfide into methylene blue, the extinction was read colorimetrically at 667 mμ. The random error of the determinations was estimated to 10–20%.

Results. The results of the sulfur analyses are presented in the Table, expressed as μg sulfur per mg dry tissue. The highest sulfur content, 6.2 ± 0.8 μg, was found in the islet tissue of the AO-mice, the corresponding value for the AOS-mice being 5.7 ± 0.8 μg ($t = 0.45$; $P > 0.05$). A considerably lower amount of sulfur was obtained for the exocrine tissue; a value of 3.2 ± 0.5 μg being measured in the AO-mice and 3.8 ± 0.5 μg in the AN-mice. In comparing the values for the endocrine and exocrine tissues in the AO-mice, it was found that sulfur was present in a significantly higher concentration in the islets ($t = 2.91$; $P \sim 0.01$).

Discussion. The sulfur concentration of the islets was considerably higher than that of the exocrine parenchyma. In addition to the sulfur of the B cells, also islet structures rich in mucopolysaccharides may have contributed to the difference. This view is supported by a previous autoradiographic study in mice showing a high uptake of S^{35}-labelled inorganic sulphate in the capillaries and connective tissue of the pancreatic islets[9].

It is generally accepted that the islet B cell granules represent a storage form of insulin. In mice with the obese-hyperglycemic syndrome, there is a very intense insulin secretion[10,11] with a lack of cytoplasmic B cell granules[12,13]. However, after 48 h starvation numerous aldehyde fuchsin positive granules appear in the B cells[14], which compose as much as about 90% of the islet cells[6,7]. It has also been shown that a regranulation of the B cells in obese-hyperglycemic mice is accompanied by an increase in histochemically demonstrable protein-bound disulfide and sulfhydryl groups in the cytoplasm of these cells[3]. It may therefore appear rather surprising that in the present investigation the total sulfur content was not significantly changed by starvation. Assuming a B cell insulin content of about 15% of the dry weight[4,5] a rough calculation shows that as much as 50% of our value for the total amount of islet sulfur may be derived from other sources than insulin. In view of the low frequency of A cells in the obese-hyperglycemic mice, their role is probably rather insignificant in this connection. A decrease in the islet mucopolysaccharides (see above) in connection with starvation might, however, well have masked a concomitant increase of insulin sulfur.

While there is scanty information about the total sulfur content of the exocrine pancreatic tissue, no previous reports seem to be available regarding the pancreatic



Isolated pancreatic islet from AO-mouse placed in 0.25 *M* sucrose and photographed in dark field illumination. A small amount of acinar tissue has not yet been removed from the lower part of the islet. × 150.

Sulfur content of isolated pancreatic islets and acinar tissue from normal and obese-hyperglycemic mice. The number of samples analysed are given within parentheses. Mean values ± S.E.

Animals	Tissue	Sulfur content μg per mg dry tissue
AN (8)	Exocrine	3.8 ± 0.5
AO (10)	Islet	6.2 ± 0.8
AO (7)	Exocrine	3.2 ± 0.5
AOS (9)	Islet	5.7 ± 0.8

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islets. A value of 112 mg sulfur per 100 g fresh organ has been found for rabbit pancreas[15]. If recalculated to dry weight, assuming a pancreatic water content of 70%[15], this figure corresponds to 3.7 μg sulfur per mg dry tissue, which compares favorably with the present values for mice.

Zusammenfassung. Schwefelmikrobestimmungen wurden an isolierten Langerhansschen Inseln fettleibiger Mäuse ausgeführt. Die Schwefelkonzentration der Inseln war nahezu zweimal so hoch wie die des exokrinen Pankreasparenchyms. Nach Nahrungsentzug wurde in den Inseln keine signifikante Veränderung des Schwefelgehaltes festgestellt.

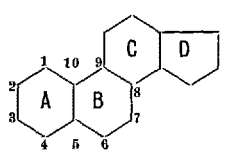
B. HELLMAN and C. HELLERSTRÖM

Histological Department, University of Uppsala (Sweden), November 6, 1962.

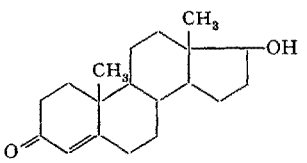
[15] F. HOPPE-SEYLER and H. THIERFELDER, *Handbuch der physiologisch- und pathologisch-chemischen Analyse* (Springer, Berlin 1953), p. 650.

Untersuchungen zur biologischen Wirksamkeit eines A-homo-Testosteronacetates

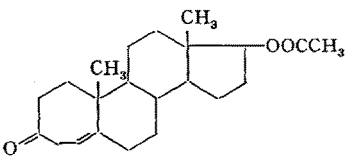
Seit kurzem besteht die Möglichkeit, in das Steranskelett durch geeignete Methoden ein weiteres C-Atom in den Ring A einzuführen und diesen damit zu einem 7-Ring zu erweitern. So haben MÜLLER et al.[1] mit Diazomethan in Position 3,4 eine Ringerweiterung bei Testosteronacetat ausführen können. Auch im amerikanischen Schrifttum[2] wurde neuerdings über diese Möglichkeit be-



Gerüst des Steroidmoleküls



Testosteron



A-homo-Testosteronacetat

richtet. Wir hatten Gelegenheit, ein in dieser Weise verändertes Testosteronacetat[3] auf seine Hemmwirkung auf die gonadotrope Partialfunktion der Hypophyse (HVL) beim Normaltier und seine Substitutionswirkung beim kastrierten Tier zu prüfen.

Material und Methodik. Die Versuche wurden an 70 ♂ Mäusen aus einheitlicher Zucht, im Gewicht um 26 g, die bei 23 ± 1°C gehalten wurden, durchgeführt. Die Ernährung erfolgte mit «altromin»-R und Wasser *ad libitum*. Folgende Versuchsgruppen wurden gebildet:

(A) 30 ♂ Mäuse, von denen 5 Tiere 10 Tage 2 mg Testosteronpropionat[4] in 0,2 ml Öl/*die* s.c., 5 Tiere 10 Tage 0,2 ml Sesamöl/*die* s.c., 5 Tiere 10 Tage 2 mg A-homo-Testosteronacetat in 0,2 ml Öl/*die* s.c. erhielten und 15 Tiere unbehandelt als Kontrollen dienten.

(B) 40 ♂ Mäuse wurden kastriert und 3 Wochen nach der Operation in Gruppen zu 10 Tieren 5 Tage lang wie unter A ausgeführt behandelt.

24 h nach der letzten Injektion, zur gleichen Tageszeit, wurden die Tiere mit $CHCl_3$ abgetötet und die Samenblasen, Präputialdrüsen sowie bei Gruppe A die Hoden und Nebenhoden sofort entnommen und in Bouinscher Lösung fixiert. Die Organe wurden in fixiertem Zustand im 80%igen Alkohol gewogen. Die Einbettung der Organe erfolgte auf übliche Weise in Paraffin. Nach Herstellung 7 μ dicker Schnitte, Färbung mit HE und HOPA (Hämalaun, Orange G, Phosphormolybdänsäure, Anilinblau). Ausmessen des Kernvolumens der Leydigschen Zwischenzellen des Hodens nach Zeichnen bei 2000facher Vergrösserung mit Hilfe eines Nomogramms. Pro Tier wurden 200 Kerne gemessen.

(A) *Hemmwirkung auf die gonadotrope Partialfunktion des HVL bei Normaltieren.* Wie aus Tabelle I hervorgeht, hatte die Behandlung einer Kontrollgruppe mit dem Lösungsmittel Öl keinen Effekt auf die Organe. Nach Zufuhr von Testosteronpropionat traten bei den Normaltieren die bekannten Veränderungen der untersuchten Organe auf. Das Hodengewicht wurde vermindert, ebenso das Kernvolumen der Leydigschen Zwischenzellen. Samenblasen und Präputialdrüsen erfuhren eine Gewichtszunahme. Gaben von A-homo-Testosteronacetat führten zu einer geringfügigen Erhöhung des Hodengewichtes. Dabei blieb das Kernvolumen der Leydigschen Zwischenzellen unbeeinflusst. Das Gewicht von Samenblasen und Präputialdrüsen wurde dagegen deutlich vermindert. Während die durchschnittliche Gewichtszunahme der unbehandelten Kontrolltiere (3,6 ± 0,4 g) und der mit Öl behandelten Kontrollen (3,8 ± 0,5 g) fast gleich war, nahmen die mit Testosteronpropionat behandelten Tiere durchschnittlich mehr zu (5,3 ± 0,4 g). Die Behandlung mit A-homo-Testosteronacetat verringerte den durchschnittlichen Gewichtszuwachs (1,9 ± 1 g) innerhalb der Versuchszeit.

(B) *Substitutionswirkung nach Kastration.* Tabelle II zeigt, dass auch hier die alleinige Behandlung mit Öl keine Veränderungen gegenüber den unbehandelten, kastrierten Kontrolltieren bewirkt. Testosteronpropionat rief eine weitgehende Restitution des Samenblasenepithels und -gewichtes sowie der Präputialdrüsenaktivität und -gewichtes hervor. A-homo-Testosteronacetat hatte keinen Einfluss auf die beiden Organe.

Diskussion der Ergebnisse. Es ist bekannt, dass Veränderungen der Substituenten am Ring A des Testosterons erheblichen Einfluss auf die androgene und anabole Wirk-

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[2] W. S. JOHNSON, M. NEEMAN, S. P. BIRKELAND und N. A. FEDORUK, J. Amer. chem. Soc. *84*, 989 (1962).

[3] Wir danken Herrn Prof. Dr. E. MÜLLER, Direktor des Chemischen Institutes der Universität Tübingen, für die Überlassung der Substanz.

[4] »Testoviron», Schering AG, Berlin.