Equal volumes of a solution of purified Substance P in gelatin were measured into a series of ampoules and freeze-dried. The amount of Substance P in one ampoule was estimated by comparison with the standard preparation (75 U/mg) by four different methods. The results are shown in the Table.

Method	Units per Ampoule
Rat uterus	< 1
Guinea-pig intestine	11.2, 13.3
Fowl rectal caecum	10
Goldfish intestine	12.2

The purified preparation had no action on the rat uterus in the concentrations used. This confirms the view (GADDUM[10]) that the action of the standard preparation on this tissue is not due to Substance P itself but to an impurity.

The results obtained by the other three methods agreed with one another within the errors of the assays. It seems likely that the action of the standard in these tests is due to Substance P itself. The results with fowl rectal caecum and guinea-pig ileum confirm similar observations by FRANZ, BOISSONNAS, and STÜRMER[11], who also found that these tests agreed with tests on rabbit blood pressure. The result with the goldfish intestine confirms the view that this tissue provides a sensitive test for Substance P (GADDUM and SZERB[12]).

Zusammenfassung. Hochgereinigte Präparate von Substanz P sind in verdünnter Lösung nicht stabil, da die aktive Substanz an Glas adsorbiert wird. Der Verlust an Wirksamkeit konnte durch Zusatz von Protein zur Lösung weitgehend verhindert werden. Die wirksame Substanz kontrahierte den Darm von Meerschweinchen, Huhn und Goldfisch, war aber am Rattenuterus fast wirkungslos.

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Histochemical Adenosine Triphosphatase in the Subcommissural Organ[1]

The subcommissural organ (S.C.O.) is a specialized part of the posterior wall of the third cerebral ventricle. No fully positive notion of the function of this organ exists, though numerous facts speak in favour of its secretory activity. Granular material considered as secretion can be demonstrated by selective histological methods in the ependymal cells of the S.C.O.

A strong activity of many histochemically demonstrable enzymes, e.g. that of acid and alkaline phosphatase and non-specific esterases, occurs in the S.C.O.[2]. These findings are taken to support the view that intense metabolic processes take place in the organ.

The purpose of our study was to investigate the eventual activity of adenosine triphosphatase in the S.C.O. and to describe its localization in this organ. The rat and the cow were taken as objects of investigation because the structure of their S.C.O. exhibits the two basic patterns of the organ. The S.C.O. of the rat is made up only of an ependymal and a hypendymal layer. In the cow, as in the other ruminants, the ependymal cells are partly located in the hypendymal region, forming hypendymal rosettes and ducts there (Figure 1).

Method. For the demonstration of adenosine triphosphatase, the method of PADYKULA and HERMAN[3] was used. Unfixed cryostat sections cut at 5–40 µ and attached to cover slips were immersed for 5–30 min at 37°C in the incubating medium with the pH 9.4. The sections were mounted in glycerine jelly.

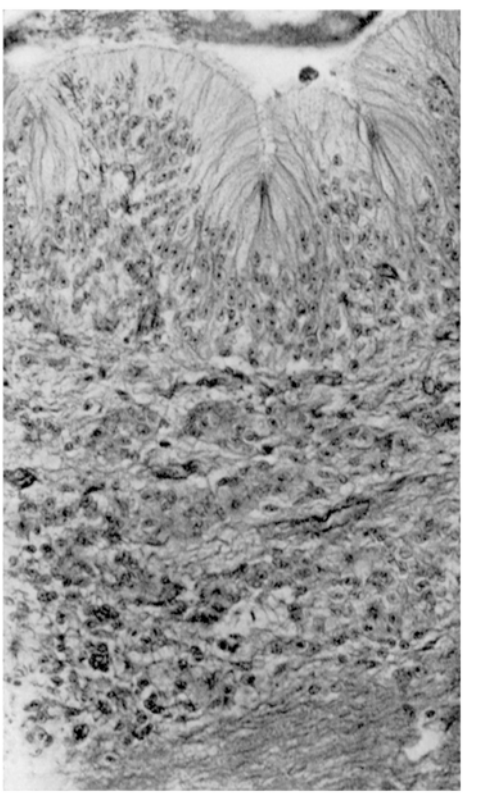

Fig. 1. Section from the subcommissural organ of the cow. The superficial ependyma is made up of tall columnar cells and the hypendyma consists of a reticular tissue and of ependymal cells forming rosettes and ducts. Below, the posterior commissure. Haematoxylin-eosin. 260 ×.

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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.

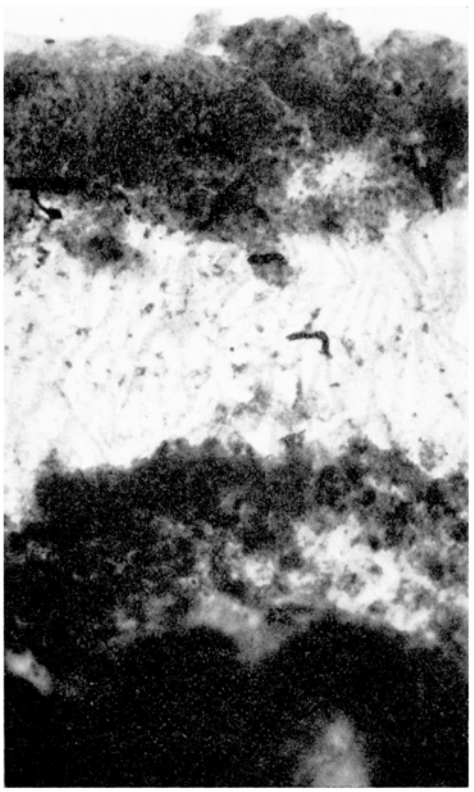

Fig. 2. Frozen section from the subcommissural organ of the cow stained for adenosine triphosphatase activity. Reaction observable in the outer ependymal layer, in the hypendymal rosettes and in the walls of the capillaries. Strong reaction in the posterior commissure. The reticular hypendymal tissue 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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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.

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