From Table IV it can also be seen that when the C-1 substituent is a methoxy group, the magnitude of the specific rotation is greater than 145°. But if a hydroxy or a methylenedioxy group is involved at C-1, the magnitude is less than 120°.

The above generalizations should be of use in the structural elucidation of new aporphine alkaloids. More importantly, they point to certain biogenetic traits in the formation of these natural products, and specifically to a relationship between structure, absolute configuration, and plant family.

Proaporphines have been clearly demonstrated to be the precursors of at least some of the aporphine alkaloids, and the trends described above may be traced back to some extent into the proaporphine series[19]. However, an insufficient number of proaporphines have been isolated so far from different sources to allow for firm generalizations.

In conclusion then, the following summary statement can be made: Naturally occurring aporphines are usually dextrorotatory and of type I. But those aporphines monosubstituted or unsubstituted in ring D, and which do not originate from the plant family Papaveraceae, generally are levorototatory and of type II. The magnitude of the specific rotation may also be useful in elucidating the substitution pattern on the aporphine skeleton[20].

Résumé. Les alcaloïdes aporphiniques substitués en positions 1, 2, 9, 10 on en 1, 2, 10, 11 sont dextrorotatoires et du type I. Mais les aporphines monosubstituées ou non substituées dans le cycle D, et qui ne parviennent pas de la famille Papaveraceae, sont généralement lévorotatoires et du genre II.

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Separate and Simultaneous Effects of Ethanol, Hypertonic Saline and Insulin on the Function of the Subcommissural Organ

The subcommissural organ (SCO) is made up of a group of ependymal cells situated above the orifice of the aqueductus cerebri. Morphological studies, including electronmicroscopical examination, have disclosed the typical characteristics of secretory cells[1]. The secretion consists of a mucopolysaccharide protein-complex which greatly resembles the neurosecretory materials of the posterior hypophysis[2]. Both secretions are revealed almost specifically by aldehyde-fuchsin staining, applied in most histochemical studies.

SCO has good humoral and neural connections: it is bathed in the cerebrospinal fluid, and it has a rich vasculature. Moreover, fibres of the autonomic nervous system are demonstrable between the secretory cells[1,3,4].

In the main, study of possible functions has proceeded by morphological means, since the SCO is small, the amount of the secretion is minimal, and under ordinary circumstances the anatomic location makes it difficult to reach. Classical ablation and extraction studies have been made, although to date the results are divergent. However, some evidence has been elicited which favours the concept that this organ might participate in regulation of the electrolyte balance[5–8].

A number of substances induce cytological changes in the cells, and these have been 'photographed' at certain moments during the experiments. For instance, hypertonic saline given for many days increases the amount of secretion[9], excessive water intake enlarges the nuclei of the SCO cells[10] and a diet deficient in sodium exercises the opposite effect, diminution of the nuclear volumes[11].

With respect to the findings mentioned it has been suggested that the cytological changes in the subcommissural cells and the electrolyte imbalances are in causal relationship, and can even be interpreted as a proof of the regulatory function exerted by SCO on the electrolyte metabolism[12].

The ingestion of ethanol inhibits liberation of the antidiuretic hormone (ADH), depresses the function of the supraoptic and paraventricular nuclei[13], and is moreover followed by plasma hyperkalemia and hyponatremia[14].

The administration of insulin leads to hypokalemia and disappearance of the aldehyde-fuchsin positive secretion in SCO within 2 weeks[15].

It is thus apparent that the substances mentioned either have, or are likely to have, an effect upon the subcommissural cells. Since they influence the electrolyte status in opposite and different ways, a clearer picture

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of the reactions of SCO might be achievable if the simultaneous effects of these substances were also tested.

Material and methods. Adult male rats of Sprague-Dawley strain were used in the experiment. After weighing, they were divided into seven groups. During the experiment, they were kept in a normal laboratory colony, and given dry pellet food and fresh tap water ad libitum. The animals received the solutions stated below to an amount of 3% of body weight as a single dose every 12 h for 14 days, through a tube inserted into the ventricle for injection of the solutions. The groups were: (1) *Ethanol group*: 16 rats (weight 180–200 g) were administered a 10% solution of ethanol; the total amount of each administration was thus 3 g ethanol/kg body weight. It had been observed that this dosage exercises an influence upon the hypothalamic neurosecretory system, and exerts a continuous effect without appreciable recovery, since some ethanol is always retained in the blood[13]. (2) *Ethanol-saline group*: 17 rats (180–200 g) were administered a solution containing 10% ethanol and 5% NaCl. (3) *Saline group*: 18 rats (170–210 g) received a 5% NaCl solution. (4) *Water or control group*: 17 rats (185–215 g) were administered normal tap water through the tube, to serve as control animals. (5) *Insulin-ethanol group*: 12 rats (180–210 g) were administered ethanol, 3 g/kg body weight, into the ventricle, and insulin, 10 IU/kg i.m. It had been found in earlier experiments that the insulin dosage employed exerts an effect on the rat hypothalamus[16]. (6) *Insulin-water group*: 11 rats (170–200 g) received tap water, instead of ethanol, and insulin as mentioned above. (7) *Saline-water group or control group*: 15 rats (190–240 g) were administered tap water into the ventricle and 0.9% NaCl 0.5 ml s.c.

After 7 days, the rats were re-weighed, and the ingested amount of fluid was corrected if necessary. On the 3rd, 7th and 14th day 2 h after ingestion, blood samples were drawn from the rats which had been administered ethanol to determine its concentration. At the end of the experiment, 2 h after the last administration of the substances, the animals were weighed and decapitated, and blood samples taken. From these, in addition to the measurement of the ethanol concentration, there were found the values of hematocrit by microcapillary technique, plasma sodium and potassium with the flame photometer, and chloride by mercuric nitrate titration. The brains were fixed in 10% neutral formaline. A block of diencephalon containing the subcommissural organ was cut out, embedded in paraffin, and sectioned serially at 7 μ. Of every 3 sections 1 was stained with aldehyde-fuchsin[17], the second with hematoxylin-eosin, and the third was discarded. The slides from test and control animals were stained at the same time. The amount of aldehyde-fuchsin positive material was estimated from photographs, taken by an automatic exposure microscopic camera, of every SCO at its largest extent by the application of an arbitrary scale from 1–5. The observers were not aware of the group of the animal to which the photographs of the sections belonged. To determine the functional enlargement, more than 1000 nuclei of SCO of each group were projected on the table, and their longest and shortest diameters were measured at a magnification of 3000. The volumes were determined according to the equation $V = 11/6\ (L\ B^2)$[18], where L is the long and B the short diameter. The calculations and the statistical analysis were carried out in the University of Helsinki Computing Centre.

Results. (A) *Effect of ethanol and saline.* During the experiment the mean weight of the rats increased in every group; the most marked rise was in the water group (mean increase 18 g) and least in the ethanol group (mean 7 g). In the ethanol-saline group, the mean increase was 12 g and in the saline group 13 g.

The blood alcohol levels in the ethanol group were about twice as high as in the group which had been administered ethanol and saline (Table I); it seems likely that saline interfered with the absorption of ethanol. In the fluid and electrolyte values, a slight difference between groups was apparent. Those given saline had a higher plasma chloride concentration 2 h after ingestion, and the groups given ethanol showed a slight tendency towards elevated plasma potassium values; however, these variations were not statistically significant, nor were those in the sodium and hematocrit values.

No marked differences between the groups were observable with respect to the amount of neurosecretory material. The ethanol and saline groups exhibited some diminution in the amount of material as compared with the water or control group (Figure 1 and 2). In the ethanol-saline group, the amount was at about the same level as in the control group (Figure 3 and 4).

Appreciable differences were discerned in the karyometric examination. The findings have been summarized in the Table II. Both in the ethanol group and in the saline group, the nuclei had shrunk remarkably. The difference from the water group was statistically highly significant ($p < 0.001$). In the ethanol-saline group, the nuclei were closer to the size of those in the water group, but smaller ($p < 0.01$).

Table I. Daily blood alcohol levels 2 h after ingestion

Group	3rd day	7th day	14th day
Ethanol	1.03% (0.52–1.60)	1.02% (0.63–1.35)	1.02% (0.66–1.66)
Ethanol saline	0.46% (0.02–1.09)	0.34% (0.00–0.90)	0.60% (0.23–1.46)
Insulin-ethanol	1.07% (0.79–1.27)	1.06% (0.45–1.73)	0.78% (0.48–1.13)

Table II. The nuclear volumes in the subcommissural organ in cubic microns (μ^3). The upper value in each column p' is the significance level of the change in volume compared to the water group and the lower value is the same to the adjacent groups. The changes are also stated per cent of the water group

Group	Nuclear volumes (1 μ^3) Mean ± S.D.	p	p 1–3
Ethanol	61.88 ± 24.16	< 0.001	0.05
	− 7.29%	< 0.001	
Ethanol saline	64.92 ± 19.87	< 0.01	
	− 2.74%	< 0.001	
Saline	60.74 ± 20.14	< 0.001	
	− 9.00%		
Water	66.75 ± 20.21		

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Micrographs from the subcommissural organs of 6 groups. The aldehydefuchsin-positive material is observable in the apical parts of the cells, and as a distinct band at the third ventricle. Reisner's fibres are visible in some pictures. × 300.

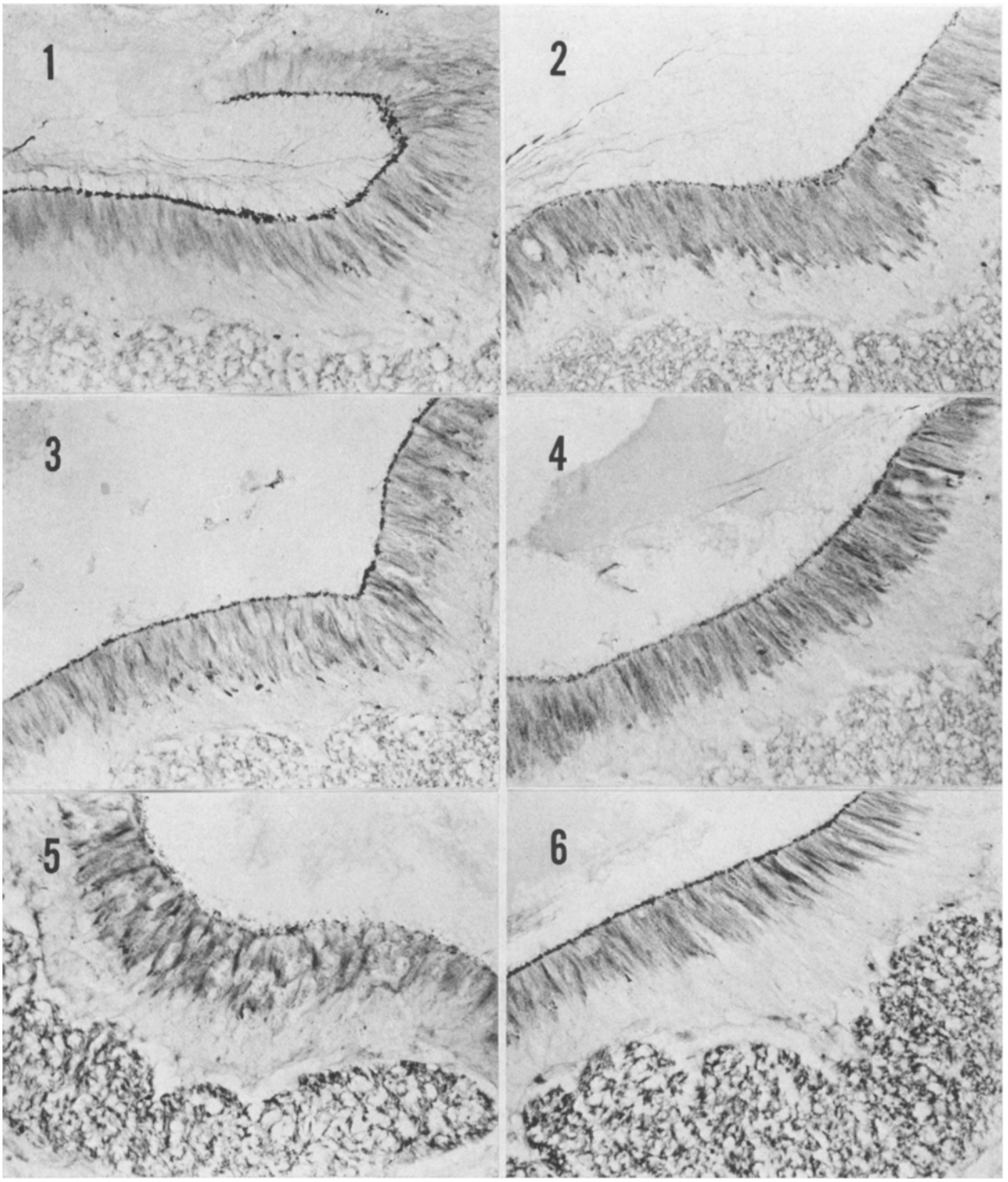

Fig. 1. SCO from the ethanol group. The amount of the secretion has diminished in the cell bodies, but a large number of granules has gathered at the ventricle.

Fig. 2. SCO from the ethanol-saline group. The amount of the material is the same as in the control group.

Fig. 3. SCO from the saline group. The cells contain less stainable material than in the control. Some cells are completely emptied.

Fig. 4. SCO from the control group. The copious secretion is evenly distributed in the cells.

Fig. 5. SCO from the insulin-ethanol group. A diminution in the secretion is evident, and vacuolization of the cells is also observable.

Fig. 6. SCO from the insulin-water group. The cells contain an amount of secretion which is slightly less than that of the control.

(*B*) *Effect of ethanol and insulin.* During the experiment, the mean weight of the rats in the 3 groups increased slightly. The blood alcohol level was about 1:1000 in the group administered ethanol and insulin (Table I).

In the group administered insulin and ethanol, a tendency was noticeable towards high, and in the groups administered insulin alone towards low plasma potassium values, although the differences were not statistically significant. A significant rise ($p < 0.01$) was apparent in the plasma sodium and chloride values in both groups. No distinct changes were remarked in the hematocrit values.

On visual estimation, less aldehyde-fuchsin positive material was evident in the apical parts of the subcommissural cells in the insulin-ethanol and insulin-water groups than in the controls (Figures 5 and 6).

Marked differences between the groups were found in the karyometric examination. The findings have been summarized in the Table II. The nuclear volumes in the insulin-water group were greater to a highly significant extent ($p < 0.001$) than in the control group. The insulin-ethanol group exhibited nuclei as great as those in the control group.

Discussion. Ethanol and saline. After administration of the agents used, there exists a tendency towards dehydration. Present information on the effect of dehydration on SCO is contradictory: GILBERT[19] has demonstrated in rats that dehydration for seven days or 2.5% hypertonic saline given for 14 days, induces an increase in the amount of stainable material in SCO cells, but KIVALO et al.[20] could not confirm this after water deprivation. As water was available ad libitum, no dehydration developed in the experiments reported here; this was also confirmed by the unelevated hematocrit values. Thus the diminution in secretory material in the groups to which saline and ethanol were administered is not related to dehydration but seems more likely to be connected with the slightly elevated plasma chloride and potassium values, respectively.

Moreover, it has been found that similar ethanol administration leads to hypertrophy of the zona glomerulosa in rat adrenals[21]. The appreciable diminution in the nuclear size in the cells of SCO can be referred to depression of the function[22]. This finding differs in part from that noted in the hypothalamic neurosecretory centers, in which ethanol reduces the cell nuclear size, but saline exerts the opposite effect[13]. These substances must also exert an influence upon mechanisms which may limit each other, as in the ethanol-saline group the combined effect was weaker than after separate administration. Analysis of the cell nuclear volumes reflects to a greater degree the changes in the SCO cells than do the alterations in the quantity of the stainable material, of which the average amount remained at control level both in the ethanol and saline and the ethanol-saline groups.

Ethanol and insulin. The highly significant increase in the nuclear volumes of the SCO cells in the insulin-water group indicates an active functional status of the organ[22]. This increase after insulin was prevented by simultaneous ethanol administration. The slight diminution in the amount of stained material in SCO might indicate an excited release of the product by insulin, if the cellular activity, as judged from the increase of nuclear volumes, is greater than normal. Ethanol did not interfere with this depletory action of insulin. One possible explanation is that hypokalemia induced by insulin activates the SCO cells. FÖLDVÁRI and PALKOVITS[11] have earlier suggested the role played by hypokalemia, on the basis of their experiments with rats living on potassium-deficient diet, which also displayed SCO nuclear enlargement. The possibility of relative insulin hypoglycemia must also be taken into account, since starvation induces a release of secretory material from mouse SCO[23].

Recent study has shown that the stainable material in SCO is composed of at least 3 different components, a protein containing a small amount of polysaccharide, a mucopolysaccharide component plus a third component which is probably glycolipoprotein[24]. It is impossible to draw clear conclusions on the basis of the relative amounts of these components. However, it seems unavoidable that many simultaneous histochemical staining methods which reveal these components must be applied if an attempt is made to find the connections with changes in carbohydrate, lipid and protein metabolism, all of which are affected by insulin.

The present findings in regard to ethanol and insulin administration are in close agreement with the concept that the functional status of SCO is altered in conjunction with electrolyte changes. However, it is necessary that the electrolyte balance can be altered locally, say in the composition of the cerebrospinal fluid, to avoid the body metabolism being affected too extensively.

Table III. The nuclear volumes in the subcommissural organ in cubic microns (μ^3). The upper value in each column p' is the significance level of the change in volume compared to the saline-water group and the lower value is the same in the adjacent groups. The changes are also stated as per cent of the saline-water group

Group	Nuclear volumes μ^3 Mean ± S.D.	p'
Insulin-ethanol	58.27 ± 17.19	0.05
	−0.61%	0.001
Insulin-water	63.54 ± 22.40	0.001
	+8.37%	
Saline-water	58.63 ± 17.86	

Zusammenfassung. Das sogenannte Subkommissuralorgan, welches sich aus Ependymzellen am Anfang des Aquaeductus cerebri aufbaut, zeigt sekretorische Elemente. Es handelt sich dabei um Mucopolysaccharide. Diese sind vergleichbar dem neurosekretorischen Material im Hinterlappen der Hypophyse. Diese Massen lassen sich durch eine Fuchsinfärbung darstellen. Männlichen Ratten wurden 10%ige und 5%ige Äthylalkohol- und Salzlösungen mit Insulin verabreicht. Insulin-Alkohol- und Insulin-Wassergruppen wiesen eine Verminderung der Fuchsin-positiven Stoffe auf. Sowohl Alkohol wie Salz, einzeln verabreicht, führen zu einer Verminderung des Zellvolumens.

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