Animals	Number of samples	Aqueous humor	Serum	Difference
Horse	4	161·4	158·2	+ 3·2
Cow	4	162·0	158·6	+ 3·4
Sow	3	160·6	159·5	− 1·4
Dog	8	159·6	157·4	+ 2·2
Rabbit	12	157·2	156·8	+ 1·4
Guinea-pig . .	12	156·3	156·8	− 0·5
Man	2	160·4	158·3	+ 2·1

tube is sealed. In the original method, the system was then left to stand at room temperature for 4 days, after which the difference in level between the two menisci was read. Since, however, the prolonged interval made it possible for the fluids to undergo chemical changes which might influence the results, the original method has been modified by NIEDERL and LEVY in such a way that the time required for equilibrium is decreased, at the same time decreasing the space to be evacuated in which evaporation and osmotic equilibrium occur.

It may be presumed that the value of exchange in the time t of the quantity of fluid evaporated, m, equals a factor α minus a quantity which is inversely proportional to the volume V. Expressed as a differential equation, this reads:

$$\frac{dm}{dt} = \alpha - \frac{\gamma}{V} m \,. \qquad (1)$$

The integral solution is:

$$m = \frac{aV}{\gamma} \left(1 - e\right)^{\frac{-\gamma}{V}} . \qquad (2)$$

Consequently, when all other conditions remain the same, decrease of the volume decreases the time needed for an equilibrium to be attained.

In my investigation, 15 mmHg was evacuated, after which the system was left to stand at room temperature for 12 h; and the difference in level between the two menisci was then read. If the two menisci were at the same level, the fluids had the same osmotic pressure; if not, they were hetero-osmotic.

Using slight magnification, one is able to demonstrate differences of 0·01 M (about 0·06 g NaCl/100 ml).

Previous to the tests of the aqueous humor, a series of tests had been made in which standard solutions of NaCl of 0·83% and of 0·98% were compared; the values for the aqueous humor were expressed in terms of isotonicity with solutions of NaCl in mM/kg H_2O.

This method, which is very simple and can be repeated at will, makes it possible to carry out determinations in quantities of aqueous humor of less than 0·1 ml.

In my present preliminary investigations, I have determined the osmotic pressure of the aqueous humor in some species of animals in relation to the osmotic pressure of the serum. The two fluids were taken from the same animal, at practically the same moment. In the Table, the results obtained in the various species examined are given.

Scrutiny of this table reveals that the hypertony of the aqueous humor over the serum is a constant finding in all the species examined, with the exception of guinea-pigs.

The fact that in guinea-pigs the aqueous humor is hypotonic with regard to the plasma is not surprising if one remembers the recent observations by DAVSON[10], who has demonstrated a difference of the ratios of concentration of bicarbonates in aqueous humor and plasma of various species of animals, and a variability of the ratio of concentration of the chlorides. The guinea-pig, in particular, shows a deficiency of anions (chlorides and bicarbonates) and it is this deficiency which explains the hypotony of the aqueous humor which makes it possible to maintain a rate of flow of the endocular fluid compatible with a relatively normal intra-ocular pressure.

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Riassunto

L'autore ha eseguito determinazioni della pressione osmotica dell'U. Acqueo e siero in alcune specie animali col metodo di NIEDERL E LEVY. In tutte le specie animali ha riscontrato sempre un'ipertonia dell'Acqueo rispetto al siero di circa 2 m/M.

Soltanto nella cavia l'acqueo è leggermente ipotonico in rapporto al siero. L'autore spiega questa eccezione con la deficienza di anioni (cloruri e bicarbonati riscontrata nell'acqueo di cavia).

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A Comparison of the Effect of Potassium Ions on the Metabolism of Retina and Brain Cortex Slices *in vitro*

It is generally accepted that potassium ions play an important role in the functional metabolism of nervous tissue[1]. Further information on the metabolic response of nervous tissue to increased K^+ concentration *in vitro* therefore appears to be desirable.

Comparing the available evidence on the metabolism of brain cortex slices and retina *in vitro*, it will be noted that an increased concentration of K^+ in glucose-containing saline brings about an increased oxygen consumption and aerobic glycolysis of incubated brain cortex slices[2], while no such response has been observed in retina[3]. The results of TERNER *et al.*[4] indicate that the same mechanism applies to the movement of K^+ in both retina and brain cortex slices. It is also known that on incubation retina releases considerable amounts of ammonia[5], in a similar way as do brain cortex slices. Experiments were published supporting the view that ammonia formation is closely connected with the functional metabolism of retina as well as of other nervous

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Oxygen consumption, ammonia formation and aerobic lactic acid formation in pig retina and guinea-pig brain cortex slices in salines of different K^+ concentrations with and without glucose. Each vessel contained 4 ml saline and tissue. The average wet weight of tissue was 192·3 ± 15·4[a] *mg retina* or 120·1 ± 10·2 mg[a] *brain cortex slices*. Each value in the Table is the average of 6 experiments (unless otherwise stated). O_2 as gaseous phase, 37°C. The results are expressed in μM/g wet weight of tissue.

	a				*b*			*c*[b]					*d*[d]		
	O_2 used (1 h)				O_2 used (4 h)			NH_3 formed (4 h)					Lactic acid formed (4 h)		
	1	2	3	4	1	2	3	4	1	2	3	4	5	3	4	5
No. of medium																
K^+ *mM*/l	6·25	131·5	6·25	131·5	6·25	131·5	6·25	131·5	6·25	131·5	6·25	131·5	TCA	6·25	131·5	TCA
Glucose *mM*/l	0	0	10	10	0	0	10	10	0	0	10	10	added at 0 time t_0	10	10	added at 0 time t_0
Pig retina	53·3 ± 5·5[a]	30·1 ± 3·4	45·4 ± 4·7	35·5 ± 2·5	130·6 ± 16·9	84·0 ± 10·1	151·5 ± 12·9	114·5 ± 9·3	20·2[c] ± 2·1	14·6[c] ± 2·2	17·5 ± 3·3	17·9 ± 2·8	6·2 ± 1·3	113·1 ± 2·8	89·4 ± 9·0	7·0 ± 1·3
Guinea-pig brain cortex slices	64·3 ± 1·7	46·0 ± 2·8	61·0 ± 4·0	78·9 ± 5·1	141·1 ± 4·6	75·8 ± 6·2	224·8 ± 9·3	252·3 ± 20·1	40·3 ± 1·6	29·8 ± 1·2	23·4 ± 2·3	26·8 ± 2·1	8·6 ± 0·9	62·6 ± 10·1	111·7 ± 15·3	10·4 ± 3·9

[a] $\sqrt{\sum \Delta^2/n(n-1)}$.

[b] A considerable part of ammonia formed during incubation of both tissues is bound by endogeneous glutamic acid as glutamine, if glucose is used as substrate[9]. Ammonia was therefore estimated in the TCA extract of the tissue after hydrolysing the extract for 75 min at 70°C[10]. The values thus present the sum of free and bound TCA-soluble ammonia. This procedure makes a direct comparison of ammonia formation in glucose-free and glucose-containing salines possible.

[c] Average of 11 experiments.

[d] In experiments recorded in section *d* 100–120 mg wet weight of tissue were used in each vessel. Each value is an average of 5 experiments.

tissue[6]. We have recently observed[7] that ammonia formation in guinea-pig brain cortex slices, incubated in glucose-free saline, was inhibited by increased concentrations of K^+. The question arose whether K^+ also inhibits ammonia formation in retina.

Experiments were performed on guinea-pig brain cortex slices and pig retina. The preparation of brain cortex slices was described earlier[7]. Pig retinas were prepared as described by TERNER *et al.*[4], with small modifications. The slices, or retinas, were then incubated in Warburg flasks. Each experiment was performed simultaneously in five media: Medium 1: Phosphate saline with a trace of bicarbonate, no substrate added[7]; Medium 2: K^+ concentration increased by completely replacing NaCl with KCl; Medium 3: same as medium 1, but 10 *mM*/l glucose added; Medium 4: same as medium 2, but 10 *mM*/l glucose added. After 4 h incubation at 37° with O_2 as the gaseous phase, the vessels were detached from the manometers and the reaction stopped by addition of 1 ml 50% trichloroacetic acid (further TCA). To each experimental series, a fifth vessel was included containing 4 ml of medium 1 and tissue, but TCA was added before the tissue was introduced (Medium 5). During the incubation oxygen utilization was measured (0·2 ml 20% KOH and a folded filter paper in the centre well of the vessels). In the TCA-extracts ammonia was estimated (for details see [7]). In a series of experiments lactic acid formed was also determined[8] after treatment of the TCA-extracts with copper lime.

The results of these experiments, recorded in the Table, permit the following conclusions:

(1) An increase of the K^+ concentration in the medium in absence of added substrate decreases oxygen consumption (Table *a* and *b*, columns 2) and ammonia formation (Table *c*, column 2) in both pig retina and guinea-pig brain cortex slices. Addition of glucose increases oxygen consumption during 4 h incubation (Table *b*, column 3), but decreases ammonia formation in both tissues studied (Table *c*, column 3).

(2) In both pig retina and guinea-pig brain cortex slices ammonia formation is inhibited by addition of glucose or by an increase of K^+ concentration in the medium. The inhibitory effects of K^+ and glucose on ammonia formation are not additive. In presence of glucose K^+ exerts no further influence on ammonia formation (Table *c*, column 4).

(3) An increase of K^+ concentration in the presence of glucose brings about a decrease of both oxygen consumption and aerobic lactic acid formation in pig retina; on the other hand, in guinea-pig brain cortex slices oxygen consumption and aerobic lactic acid formation are increased under these conditions (Table *d*).

Thus both tissues show a marked difference in their metabolic response to increased K^+ concentration as far as oxygen utilization and aerobic lactic acid formation are concerned, but behave similarly as far as ammonia formation is concerned.

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Zusammenfassung

Der Einfluss einer erhöhten Kalium-Ionenkonzentration auf den *in vitro*-Metabolismus von Schweine-Retina und Gehirnrindenschnitten des Meerschweinchens wird verglichen. Die höhere Kalium-Ionenkonzentration im Medium bewirkt im ersten Falle eine Verminderung, im zweiten dagegen eine Steigerung von Sauerstoffaufnahme und anaerober Milchsäurebildung. Bei beiden Geweben wird durch die vermehrte Kalium-Ionenkonzentration die Bildung von Ammoniak gehemmt.

Corticotrophin-Releasing Action of Adrenaline, Serotonin and Pitressin

There is much evidence that the secretion of ACTH is controlled by a hypothalamic neuro-humor. The nature of this corticotrophin-releasing factor (CRF) remains obscure. Several substances have been suggested as possible transmitters. Among these are adrenaline[1], serotonin[2], and vasopressin[3] or a vasopressin-contaminant[4].

As the CRF must be able to stimulate the pituitary gland directly, the effect of these substances was investigated in animals in which hypothalamic functions were inhibited.

Albino rats were injected with 30 µg adrenaline intraperitoneally, 25 µg serotonin picrate[5], 0·2 I.U. Pitressin (Parke, Davis) or 0·2 ml saline intravenously per 100 g body-weight respectively. The release of ACTH was measured by the adrenal ascorbic acid depletion; the rats were sacrificed by decapitation 1½ h after the administration of the compounds, and the ascorbic acid content of both adrenals was determined according to SAYERS *et al.*[6].

The first experiment was carried out with normal male rats weighing 150–180 g. As may be seen from the Table, the adrenal ascorbic acid content of rats treated with any of the compounds differs statistically significant from that of the saline-treated rats.

In the second experiment, with male rats weighing 150–180 g, a corticoid was used as a hypothalamic blocking agent. 5 mg Prednisolone[7] per 100 g body-weight was injected subcutaneously 24 h prior to the administration of the compounds under investigation. The Table demonstrates that the effect of both adrenaline and serotonin on the adrenal ascorbic acid content is inhibited under these conditions, whereas Pitressin still induces a statistically significant ascorbic acid decrease.

Treatment	Adrenal ascorbic acid content		
	None	Prednisolone	Lesioned
Pitressin . .	274 (± 6·5)*†	438(±10·2)†	316(±16·6)†
Adrenaline .	294 (± 8·9)†	514(±16·2)	380(±18·0)
Serotonin . .	295 (± 10·8)*†	511(±10·6)	401(±17·4)
Saline . . .	349 (± 10·0)	525(± 7·2)	396(±15·0)

The data are given in mg ascorbic acid / 100 g adrenal tissue, with the standard error of the mean between brackets.

† statistically significant decrease as compared with saline-treated controls.

* in these cases 13 rats are used; in all other cases 14.

The last experiment was performed in hypothalamic-lesioned rats. In this case female rats weighing 190–210 g were used. Electrolytic lesions were made by means of a modified stereotactic instrument after GREER *et al.*[8]. A detailed description of the lesion technique is given elsewhere[9]. Lesions were placed in the posterior part of the eminentia mediana, interrupting the hypothalamo-hypophysial connections.

As is demonstrated in the Table, serotonin and adrenaline again failed to show ACTH-releasing activity in this experiment. On the other hand Pitressin is able to activate the hypophysio-adrenal axis.

The results reported here do not support the view that either adrenaline or serotonin would exert a specific action in the mechanism of the ACTH secretion from the adenohypophysis. The demonstration of the pituitary activation by Pitressin in Prednisolone-treated and hypothalamic-lesioned rats is in accordance with the findings of several authors[10]. Whether this effect is due to the true vasopressin or to another polypeptide contaminating Pitressin is not decided by these experiments.

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Zusammenfassung

Der Einfluss von Serotonin, Adrenalin und Pitressin auf den Mechanismus der ACTH-Ausschüttung durch den Hypophysenvorderlappen wurde unter verschiedenen experimentellen Bedingungen untersucht.

Bei normalen Ratten zeigten alle geprüften Pharmaka auf die Adenohypophyse eine stimulierende Wirkung, welche nach der Abnahme des suprarenalen Askorbinsäuregehaltes beurteilt wurde.

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