Zusammenfassung

Mittels Papierchromatographie wurde das Bild der freien Aminosäuren der Netzhaut und des Cornea-Epithels des Rindes untersucht und mit den Aminosäuren des Hydrolysats des gesamten Gewebes in Beziehung gebracht. Die Ergebnisse zeigen, dass das Bild der freien Aminosäuren ein charakteristischer Zug eines bestimmten Gewebes ist, obwohl er nicht ganz genau dessen Zusammensetzung an Aminosäuren wiedergibt.

Diurnal Changes in the Brain Glycogen

As changes in the concentration of brain glycogen parallel several altered states of behaviour[1], the question arises what connection this brain metabolite has to autonomic functions which are regulated at the highest level. In the present report, the rhythms of sleep and wakefulness were chosen as representative of these functions.

In 123 rats the concentration of brain glycogen was determined in 6-h intervals in five topographical regions of the brain. The same method was used as described in a preceding paper[2].

Diurnal changes of glycogen in the cerebral cortex and the diencephalon

Astronomic time	Glycogen (mg/100 g of wet weight)*			
	Cerebral cortex	*n*	Diencephalon	*n*
0	91 ± 5·2	31	117 ± 3·8	28
6	86 ± 4·6	34	131 ± 6·2	25
12	89 ± 5·4	28	135 ± 4·2	26
18	90 ± 4·1	30	127 ± 5·1	28

* Mean ± S. E.

In the Table the characteristic results of these experiments are given. From these it follows that, while there are no significant periodic changes in the cerebral cortex, the mesencephalon, cerebellum, and medulla oblongata, there is a significant difference in the diencephalon ($P < 0{\cdot}01$ between the interval 0 and 12).

The maximal concentration of glycogen in the diencephalon coincides with the period during which there is a maximal readiness of the rat to the cerebral inhibitory state[3].

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Zusammenfassung

In 6stündigen Intervallen wurde bei der Ratte die Konzentration des Glykogens in der Hirnrinde sowie im Diencephalon, Mesencephalon, Cerebellum und im verlängerten Mark festgestellt. Innerhalb von 24 h tritt im Diencephalon – und nur in diesem – periodisch eine signifikante Veränderung der Glykogen-Konzentration ein.

[1] M. R. A. CHANCE and D. C. YAXLEY, J. exp. Biol. *27*, 311 (1950). – N. SHIMIZU and Z. KUBO, J. Neuropath. exp. Neurol. *16*, 40 (1957).
[2] D. SVORAD, Nature *181*, 775 (1958).
[3] D. SVORAD, Physiol. Bohemoslov. (1958), in press.

Effect of Aldosterone and Hydrocortisone on Sodium in Red Cells

Studies with aldosterone in the rat have recently led us to the theoretical conclusion that this steroid, and perhaps related ones as well, can act by conditioning sodium flux across cell membranes. We have suggested that the net effect favors an increase in intracellular sodium, possibly by hindering sodium transport (efflux[1]). The red cell seemed particularly appropriate for a direct experimental approach to this postulate. GLYNN also predictively considered this but was unable to demonstrate any effect[2]. He used red cells in a buffered medium, however. It seemed to us that the problem could not be definitively settled unless plasma were used as medium since quite possibly a cofactor or carrier is required. Further, it seemed desirable to subject the cells to forcing procedures favoring separately either influx or efflux. Accordingly, our experiments were designed to use whole blood and to follow the changes in plasma [Na] and [K] during refrigeration or subsequent rewarming. In view of our inexperience in the difficult field of red cell experimentation and of the inherent variability of whole blood studies, experiments were freely repeated. Well defined effects were demonstrated both with aldosterone and with hydrocortisone.

Methods. – Fresh blood from student volunteers was collected in heparin (0·2 ml/10 ml blood), centrifuged, and the buffy coat removed. The blood was then reconstituted. It was divided into 5 ml aliquots and each was sampled separately for the determination of plasma Na and K and of hematocrit. The aliquots were then transferred to prepared tubes containing the steroid in 0·1 ml of vehicle, or the vehicle alone. In cooling experiments the tubes were placed in a refrigerator at 4°C and sampled in the cold 2, 4, 6 or 24 h later. While in the refrigerator, they were inverted gently every half hour throughout the day. In rewarming experiments, untreated 5 ml aliquots were first refrigerated for 24 h and then sampled separately in the cold before transfer to fresh tubes containing the steroid or vehicle. Sampling was again carried out after 0·5, 1, 2, and 3 h of rewarming at 37°C. Aldosterone racemate in ethyl alcohol and hydrocortisone acetate in saline were used.

Calculations were based on the change in each datum from its own base value taken as zero. Regression coefficients were determined where applicable[3]. Further details are given with each experiment.

Results.—Effect of aldosterone on Na, K, and hematocrit during rewarming. Aliquots (5 ml) containing 1, 1·4, 1·8, and 2·0 µg/ml of aldosterone racemate, or the vehicle alone, were rewarmed and sampled as described. Five separate runs were carried out. The figure presents the essential findings. Aldosterone clearly depresses the rate of Na extrusion during rewarming. This is preceded during the first half hour by a fall in plasma [Na] which is most prominent at lower doses. The proportionality of the effect is shown by the decreasing values of the regression coefficient with increasing dose (Table I). The effects on Na are not accompanied by similar changes in K or hematocrit; these show the normal patterns of rewarming.

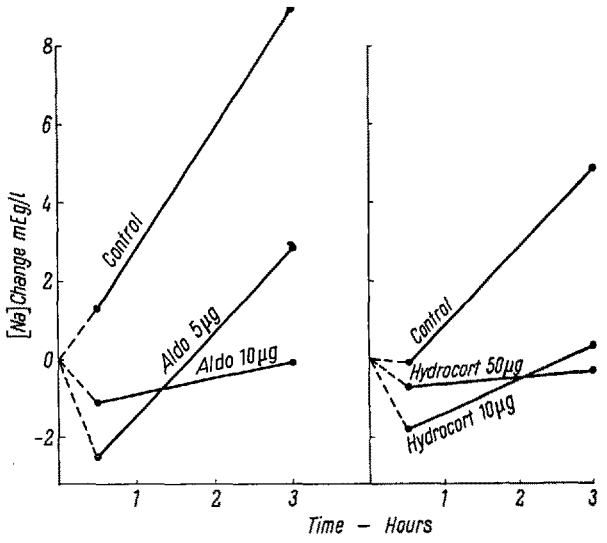
[1] S. M. FRIEDMAN, C. L. FRIEDMAN, and M. NAKASHIMA, Amer. J. Physiol. (in press for Dec. 1958).
[2] I. M. GLYNN, J. Physiol. *136*, 148 (1957).
[3] R. A. FISHER, *Statistical Methods for Research Workers*, 12th ed. (Oliver and Boyd, Edinburgh 1954).

Table I. Regression statistics for aldosterone and hydrocortisone treated red cells in plasma compared with untreated controls

Treatment	Δ[Na] mEq/l a	Δ[Na] mEq/l *b	Δ[Na] mEq/l 0·5 h	Δ[K] mEq/l a	Δ[K] mEq/l b	Δ[K] mEq/l 0·5 h	Δ Hematocrit (%) a	Δ Hematocrit (%) b	Δ Hematocrit (%) 0·5 h
Control	+4·31	+2·64 ± 0·51	+1·34	−0·99	−0·70 ± 0·22	−0·20	−4·1	−0·54 ± 0·48	−3·5
Aldosterone, 1 μg/ml	−0·03	+2·18 ± 0·27	−2·49	−1·10	−0·68 ± 0·20	−0·34	−4·5	−0·56 ± 0·46	−3·9
Aldosterone, 1·4 μg/ml	−0·90	+1·96 ± 1·27	−3·32	−1·25	−0·65 ± 0·20	−0·51	−4·5	−0·65 ± 0·53	−3·7
Aldosterone, 1·8 μg/ml	+0·68	+1·19 ± 0·84	−0·66	−1·30	−0·51 ± 0·24	−0·73	−5·0	+0·15 ± 1·46	−4·8
Aldosterone, 2·0 μg/ml	−0·68	+0·40 ± 1·32	−1·14	−1·05	−0·61 ± 0·16	−0·36	−3·7	−0·59 ± 0·53	−3·1
Control	+2·06	+1·96 ± 1·06	−0·14	−0·74	−0·45 ± 0·07	−0·23	−5·6	−0·45 ± 0·45	−5·1
Hydrocortisone, 0·2 μg/ml	−0·54	+1·38 ± 0·67	−2·09	−0·72	−0·47 ± 0·06	−0·19	−5·4	−0·69 ± 0·42	−4·6
Hydrocortisone, 2·0 μg/ml	−0·86	+0·84 ± 0·45	−1·81	−0·91	−0·48 ± 0·11	−0·36	−5·8	−0·79 ± 0·38	−4·9
Hydrocortisone, 5·0 μg/ml	−0·58	+0·16 ± 0·95	−0·76	−0·78	−0·49 ± 0·08	−0·22	−5·4	−0·83 ± 0·39	−4·5

a = mean of the dependent variate; b ± coefficient of regression and standard error; 0·5 h = calculated value of the variate at 0·5 h.
* Significant shift of the coefficient of regression occurs in this column.

Effect of hydrocortisone on Na, K, and hematocrit during rewarming. Aliquots (5 ml) containing 0·2, 2·0, or 10·0 μg/ml of hydrocortisone acetate, or the vehicle alone, were rewarmed and sampled as described. Five separate runs



were carried out. The Figure presents the essential findings. Hydrocortisone also produced a depression of the rate of Na extrusion preceded by a sharp fall in plasma [Na]. There was a similar proportionality to dose (Table I). Plasma [K] followed the control pattern but there was a suggestion that the fall in hematocrit with time might be steeper as the dose of hydrocortisone increased.

Effect of aldosterone or hydrocortisone on Na, K, and hematocrit during 6 h of cooling. Experiments carried out as described using the same doses of the steroids failed to show any effects during a 6-h cooling period.

Effect of aldosterone on Na, K, and hematocrit during 24 h of cooling. 20 ml of whole blood free of buffy coat was divided into two equal aliquots after a preliminary sample was taken for analysis. To one aliquot was added 5 μg of aldosterone, to the other the vehicle only. Both were then refrigerated for 24 h. Samples were taken in the cold at 6 h and at 24 h. From each aliquot, three separate samples were taken and each analyzed in triplicate. This ninefold analysis permitted a reasonably accurate statement of concentration. Four identical runs were carried out (Table II). Clearly, in all 4 runs the rate of Na accumulation by the cells was depressed in the treated samples. It should be noted that the dose used was lower than in the preceding experiments. Unfortunately, the degree of difference between control and test aliquots is too close to the intrinsic method error for absolute confidence and we are at present unable to extend the series because of a seasonal shortage of donors.

Discussion.—These experiments show that both aldosterone and hydrocortisone affect Na flux. Further, the doses used are reasonably small in comparison with doses of desoxycorticosterone which have yielded positive results[4]. Recently, GLYNN has demonstrated that cardiac glycosides in very low concentrations inhibit the movement of sodium and potassium ions across the human red cell membrane, probably by an action on the transport mechanism itself rather than on the energy supply to the pump[4]. He reasoned that aldosterone is sufficiently re-

[4] E. S. JONES, Exper. *14*, 72 (1958).

Table II. The effect of 0·5 μg/ml of aldosterone on plasma [Na] during 24 h cooling of human whole blood. Note the consistent trend for [Na] to remain higher in test groups than in controls. Plasma [Na] mEq/l

	0 h	6 h		24 h	
	Test	Control	Test	Control	Test
Run 1	141·2 ± 0·9	134·4 ± 0·6	137·4 ± 1·7	132·0 ± 0·3	134·4 ± 1·4
Run 2	146·4 ± 2·0	137·2 ± 0·8	143·2 ± 1·8	133·4 ± 1·5	135·0 ± 0·9
Run 3	142·0 ± 0·8	141·6 ± 1·2	142.4 ± 2·4	133·4 ± 0·2	140·8 ± 0·8
Run 4	142·3 ± 1·1	130·5 ± 0·7	139·8 ± 3·2	130·3 ± 0·4	133·5 ± 0·7

± = standard error of ninefold analysis

lated both chemically and functionally to produce similar effects. He could not, however, demonstrate any effect with doses similar to ours (10^{-5} or 10^{-6} g/ml). The major difference between these experiments and ours is our use of plasma as suspending medium. The longer duration of our experiments seems a less likely explanation for the divergence.

The gross measurement of sodium concentration in the medium does not permit definitive conclusions regarding the site of action of these adrenal cortical steroids. The results are, however, quite consistent with the view which we have expressed elsewhere that aldosterone hinders sodium efflux[1]. Judging by GLYNN's analysis, this may well represent an action on the transport mechanism itself.

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Zusammenfassung

Natrium- und Kalium-Plasmakonzentrationen und Hamatokritwerte wurden in menschlichem Blut nach Beifügung von kleinen Mengen Aldosteron nach 2-, 4-, 6- oder 24stündiger Kühlung oder nach $^1/_2$-, 1-, 2- oder 3stündiger Erwärmung gemessen. Aldosteron vermindert die Plasma-Natriumzuwachsrate bei Erwärmung und anscheinend auch die Plasma-Natriumverminderungsrate bei Kühlung. Hydrokortison ergab das gleiche Resultat bei Erwärmung, während seine Wirkung bei Kühlung noch nicht abschliessend untersucht wurde.

Some Factors Modifying the Action of Chlorpromazine upon the O_2 Uptake of Brain Homogenates *in vitro*

Studying the direct action of chlorpromazine on isolated tissues[1], we have investigated the *in vitro* effect of Largactil upon the respiration of brain homogenates (albino rats) under various experimental conditions, i.e. in the medium without glucose, in the calcium-free suspension fluid, and on homogenates stimulated with 2,4-dinitrophenol.

The O_2 uptake was estimated by direct Warburg technique, at the temperature of 37°C and in pure oxygen. Medium: phosphate buffered Krebs Ringer solution with or without 0·2% glucose and calcium-free solution (100 ml 0·154 *M* NaCl, 4 ml 0·154 *M* KCl, 1 ml 0·154 *M* $MgSO_4 \cdot 7\,H_2O$, and 30 ml of phosphate buffer) with 0·2% glucose.

Changes in metabolic rate after addition of various concentrations of Largactil are given in Table I.

When Largactil was added to the Krebs Ringer solution without glucose, no significant changes in the O_2 uptake were observed (Table II).

The depressive action of Largactil was diminished in the same way in the calcium-free solution (Table II).

Table I

Oxygen uptake at various concentrations of Largactil in brain homogenates of albino rats. Medium: Buffered Krebs Ringer solution with 0·2% glucose. The inhibition at all concentrations is significant

No. of experiment	Treatment	µl O_2/100 mg wet w./h		Inhibition in %
		M	S.D.	
15	Control	147·04	± 15·15	
	Largactil/ml:			
8	1 µg	128·66	± 8·6	− 12·5
6	5 µg	118·10	± 4·3	− 19·7
13	10 µg	108·29	± 10·1	− 26·4
6	50 µg	108·29	± 16·5	− 26·3
7	100 µg	106·65	± 8·65	− 27·5
5	200 µg	76·34	± 14·5	− 48·2

Table II

Oxygen uptake of brain homogenates after Largactil in Krebs Ringer solution without glucose$^+$ and in calcium-free solution^{++} with 0·2% glucose. The inhibition is not significant

No. of experiment	Treatment	µl O_2/100 mg wet w./h		Inhibition in %
		M	S.D.	
6	Control	108·28	± 4·64	
5	Largactil (10 µg/ml)	103·50	± 9·34	− 4·42$^+$
5	Control	180·65	± 19·1	
5	Largactil (10 µg/ml)	172·20	± 17·2	− 4·68^{++}

Table III

The inhibition of the O_2 uptake after Largactil in brain homogenates treated with 2,4-dinitrophenol (DNP). Medium: Buffered Krebs Ringer solution with 0·2% glucose. The inhibition is significant

No. of experiment	Treatment	µl O_2/100 mg wet w./h		Inhibition in %
		M	S.D.	
9	DNP (20 µg/ml)	221·94	± 36·64	
9	DNP (20 µg/ml) + Largactil (10 µg/ml)	185·88	± 28·70	− 16·2

In further experiments, Largactil exhibited an antagonizing action upon the increased metabolic activity of brain homogenates stimulated with 2,4-dinitrophenol (Table III).

The direct influence of relatively low doses of Largactil upon the respiration of brain homogenates was confirmed. This agrees with the work of GÄNSHIRT and BRILMAYER[2] who have found a significant decrease of respiration activity in brain homogenates of white mice after Megaphen at concentration of 10^{-6} g/ml. On the other hand, DECSI[3], considering the direct action of chlorpromazine upon the cerebral tissue emphasizes in his recent studies that the inhibition of phosphorylations seems to be prevailing and can be demonstrated even at the dose which does not affect the oxygen uptake. The decrease of the oxygen consumption, according to this further findings, does not

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[2] H. GÄNSHIRT and H. BRILMAYER, Arch. int. Pharmacodyn. *98*, 467 (1954).

[3] L. DECSI, Acta physiol. Hung. *10*, 387 (1956). – L. DECSI and J. HEIDT, Acta physiol. Hung. *13*, 183 (1958).