Results, as can be observed in the Table, indicate that basal cyclic AMP levels vary between 16 to 165 pmoles/mg sperm protein. These variations in cyclic AMP levels are comparable to those observed by GRAY, HARDMAN, HAMMER, HOOS and SUTHERLAND[9]. Further, our studies demonstrate that sperm cAMP levels increased several fold on addition of spermine (2.9 mM to 13.4 mM). The results presented clearly demonstrate that the physiological concentration of spermine enhances the cAMP levels considerably. GARBERS, FIRST and LARDY[6] and HICKS et al.[10] have observed that cyclic nucleotides regulate spermatozoal motility and metabolism. They have also shown that cAMP increase the oxidation of lactate, succinate and citrate by human spermatozoa. Further, the increase in oxidation metabolism resulted in increased spermatozoal motility[11]. TASH and MANN[12] have shown that concentration of cAMP in spermatozoa represents a very accurate and sensitive indicator of sperm activity. According to our findings, polyamine can activate cAMP levels of spermatozoa which provides the basis for the observation of TABOR and TABOR[2], who reported activation of spermatozoal motility.

Presence of polyamines and enzymes involved in polyamine biosynthesis-ornithine decarboxylase in human cervical mucus have been detected by us (unpublished observation). RUSSELL et al.[13] have reported the presence of ornithine decarboxylase activity in the rat uterus. Further, these workers have shown that the enzyme level increases on administration of estradiol. If human spermatozoa undergo capacitation, as do the spermatozoa of hamster, rabbit etc., then polyamines present in the female genital tract would have an important role to play in sperm capacitation, by increasing the spermatozoal cAMP levels. The mechanism by which polyamine activates adenyl cyclase reamins to be elucidated.

Zusammenfassung. Zusätze physiologischer Sperminkonzentrationen (2–14 mM) zu menschlichen Spermiensuspensionen bewirken eine Steigerung der Adenylcyclase-Aktivität, wie sie durch die vermehrte Bildung von cAMP aus ATP angezeigt wird.

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Cerebral Uptake of Noradrenaline in vitro; Occurence of Different Uptake Systems and Effect of Partial External Sodium Substitution

Exogenous noradrenaline (NA) is actively accumulated into cerebral cortex slices against a concentration gradient[1,2]. NA is inactivated by re-uptake into the presynaptic endings after its release from synapses. SNYDER et al.[3] have studied NA accumulation by different brain structures incubated in oxygenated physiological media. They found that there was only a single NA uptake system with different kinetic parameters for different structures, except in the case of the cerebellum which did not show saturable accumulation[3].

Cerebral tissue have different affinities for the uptake of dopamine[4], serotonin[5], γ-aminobutyric acid[6] and choline[7]. We have studied NA transport in cerebral cortex slices with a larger range of concentrations than those used by SNYDER et al.[3].

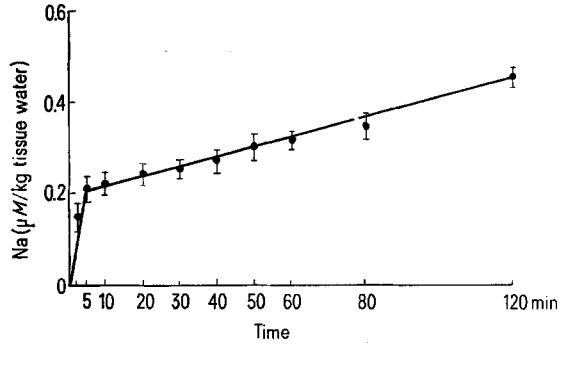

Fig. 1. Kinetics of the accumulation of DL-^{3}H-NA at a concentration of 0.2 μM/l at 37 °C in a normal Krebs Ringer medium. Each point is the mean of 10 slices, with the vertical bars representing one standard deviation.

We also studied the effects of Na^+ deficient medium on NA cerebral transport as the uptake of these other compounds is dependent upon the concentration of ions in the medium[8].

Experimental procedure. Male Wistar rats, weighing 200–250 g were used. The preparation and incubation of the cerebral slices were carried out as described previously[9,10]. Slices (60–70 mg) were pre-incubated for 30 min in Krebs-Ringer bicarbonate saline which was also used for incubation; it had the following composition, (mM), NaCl, 124; KCl, 5; KH_2PO_4, 1.24, $MgSO_4$, 1.3; $CaCl_2$, 2.8; $NaHCO^3$, 26; glucose, 10; the pH was kept at 7.4 by continuous saturation with 95% O_2: 5% CO_2 mixture. When Na^+ deficient solutions were used, the NaCl and $NaHCO_3$ were replaced by choline chlorydrate and choline bicarbonate, respectively (Sigma Co., St. Louis, USA).

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Immediately after the end of preincubation, the slices were transferred for 5 min to an incubation medium containing in addition DL-^{3}H-NA (500–5000 mCi/mM) (The Radiochemical Centre, Amersham, U.K.) previously purified by amberlite CG 50 to remove radiolysis products. DL-^{3}H-NA was used with a final activity of 0.05 mCi/l. Different concentrations of non-radioactive NA were added to the medium, as indicated in the figures.

After incubation, the slices were quickly dried on scintered glass, homogenized in 2.0 ml of trichloracetic acid (6%) and centrifuged; 1.0 ml of the supernatant was mixed with 10.0 ml of Bray's solution[11] and counted for 10 min with a Tri Carb 2003 Packard Liquid Scintillation Spectrometer, with external standards to correct for quenching. The tissue water was calculated as the difference between the final incubated weight and the weight of the tissue after dehydration for 12 h at 105 °C. The concentrations of tissue water were 86.6% at 37 °C and 89.2% at 0.5 °C in normal incubation medium, and 87.6 at 37 °C and 91.3% at 0.5 °C in Na^+ deficient medium. The rate of NA uptake was expressed on the basis of tissue water as the final incubated tissue weight is proportional to the tissue water.

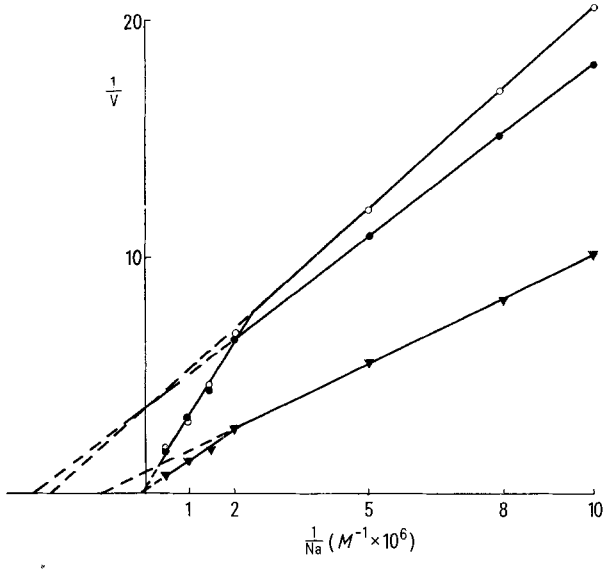

Fig. 2. Lineweaver-Burk plot of the initial rate of uptake at 37 °C (5 min) of DL-^{3}H-NA in the concentration range of 20 μM/l to 0.1 μM/l. ▲, The tissue was incubated in normal Krebs Ringer solution. The plot makes no correction for simple diffusion. ●, A similar plot of tissue in normal Krebs Ringer solution, but corrected for simple diffusion. ⊙, Tissue incubated in a modified Krebs-Ringer solution with 75 mM Na^+ and 75 mM choline. The plot is corrected for simple diffusion. The rate of uptake (V) is expressed in μM/kg tissue water/min. Each point is the mean of 10 slices.

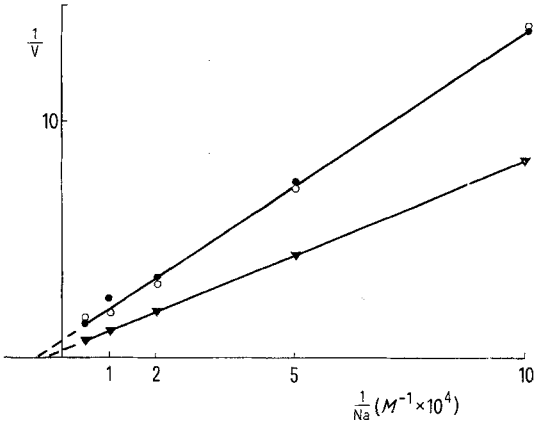

Fig. 3. Lineweaver-Burk plot of the initial rate of uptake at 37 °C (5 min) of DL ^{3}H-NA in the concentration range of 0.2 mM to 0.01 mM, ▲, ●, and ○ are the same as for Figure 2. The rate of uptake (V) is expressed in 0.1 mM/kg tissue water/min. Each point is the mean of 10 slices.

Results. The accumulation of NA by the tissue in the presence of a concentration in the medium of 0.2 μmol/l was examined at 37 °C at various incubation times (Figure 1). The initial rapid uptake was linear for 5 min and was followed by a slower rate for a further 120 min.

The initial rates of uptake for 5 min at different medium Na concentrations are shown in Lineweaver Burk plots. The results are expressed with and without corrections for simple diffusion obtained from similar incubations at 0.5 °C for each concentration.

1. If no correction was made for simple diffusion, the uptake was characterized by 2 rates. The NA uptake system had a low affinity when the initial concentration of NA in the medium was between 0.01 mM/l and 0.20 mM/l (Figure 3) with an apparent Km of 0.4 mM/l and a V_{max} of 0.08 mM/l/min. The tissue showed a high affinity with low concentrations of NA (Figure 2) (0.1 μM/l to 2 μM/l) to an apparent Km of 1.0 μM/l and a V_{max} of 0.18 μM/l/min. The affinity of this second system was 400 times higher than that of the first one.

2. When correction was made for simple diffusion, there were apparently significant changes in the parameter of uptake system. For the low affinity system, the apparent Km was now 0.2 mM/l and the V_{max} was 0.014 mM/l/min. For the high affinity system, the apparent Km was 0.4 μM/l and the V_{max} was 0.27 μM/l/min.

3. Incubation in a low Na^+ medium did not change the kinetic parameters for the low affinity system. In contrast, the apparent Km for the high affinity system was increased to 0.5 μM/l although the V_{max} had a value corrected for diffusion which was not significantly different.

Discussion. To the best of our knowledge, a dual brain uptake system for NA in vitro has not previously been reported, probably due to the narrow range of concentrations previously studied[3].

The kinetic parameters of the high affinity system are similar to the values reported for the single uptake system by SNYDER[3,12] for brain slices, brain homogenates[4] and brain synaptosomal fractions[13].

Some membrane active transport systems require the presence of Na^+ ions[10,14]. The iso-osmotic substitution of this action by Li^+ or K^+ strongly decreases NA accumulation by cardiac slices[15] and decreases the NA and serotonin uptake by brain synaptosomal fractions[8].

In our experimental conditions, the selective sensitivity of the high affinity NA uptake system to a low Na^+ medium, parallels the results of YAMAMURA and SNYDER[7] who have recently reported differential effects of Na^+-free media on the two cerebral uptake systems for choline. Likewise, the kinetic parameters of γ-aminobutyric acid uptake into synaptosomes are simultaneously but differentially affected by low Na^+ concentration in the

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medium[16]. In view of these findings, it is possible to suggest an allosteric function for the external Na^+ in the transport systems for all these molecules and for NA.

Résumé. L'étude de l'accumulation de la noradrénaline marquée par des coupes de cortex cérébral de rat, montre l'existence de deux systèmes de capture. Seul le Km du système à haute affinité est affecté par l'exclusion partielle des ions Na^+ du milieu d'incubation (Na^+ = 75 m*M*) par la choline.

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Variation in the Total Content of Alkaline RNAse in Mouse Lymphocytes from Different Organs

Wide variations in the alkaline ribonuclease (RNAse) activity of different populations of lymphocytes were recently reported by MANSSON et al.[1] in a group of 20 patients with chronic lymphocytic leukemia in whom the total RNAse content per cell varied by a factor of 20. In experimental animals, the content of alkaline ribonuclease of homogenates from thymus, spleen and lymph nodes is raised many times following a variety of stimuli which include irradiation by X-rays of the whole body[2,3], irradiation of the head only[3,4], injection of cortisone and other hormones[5,6] and antigenic stimulation[7,8]. In our studies, the value of RNAse/mg of DNA in control animals was higher for spleen than for thymus[3,6] and it seemed that there were variations in RNAse content between different populations of lymphoid cells. KRAFT and SHORTMAN[9] had reported that peritoneal cells of rats contained much more alkaline RNAse than lymphocytes from lymph nodes and spleen, and they attributed this to the presence of macrophages. However, the results of the present investigation show that lymphocytes from the peritoneal cell population have higher content of RNAse than the macrophage, and that by comparing the RNAse content of adherent and non-adherent cells from the thymus, lymph nodes and peritoneal cell population, of normal mice and mice treated with cortisone, subpopulation of lymphocytes which are exceptionally rich in RNAse were identified. Previously we[10] found that, at the end of the regenerative phase following partial hepatectomy, the RNAse content of livers rose sharply for a few days, and we proposed that RNAse was involved in the control of cell division. A similar suggestion was subsequently made by KRAFT and SHORTMAN[9], and it is tempting to speculate that the difference in the RNAse content of lymphocytes from different sites may also be related to the time at which they had undergone cell division.

Materials and methods. Random bred mice, 10–12 weeks old, were used. Peritoneal cells, thymuses and mesenteric lymph nodes were pooled from 5 animals per experiment. In the cortisone-treated groups, 5 mg of cortisone/mouse (Frederiksberg, Copenhagen) were injected s.c. either 1, 2 or 3 days prior to the removal of the lymphoid cells. Peritoneal cells were obtained without prior stimulation[11,12]. Peritoneal thymus and mesenteric lymph nodes cell suspensions were prepared at 10^6 cells/ml. Separation of adherent and non-adherent cells was performed according to EVANS and ALEXANDER[11]. 2 ml of the suspension of cells derived from peritoneum, thymus or lymph nodes were cultured either in a 3.5 × 6 mm Falcon plastic petri dish or in a siliconized glass tube in medium M199. After the separation, these suspensions were diluted 1:1 with distilled water, and the estimation of total RNAse content of cells was carried out as described previously[8].

Results and discussion. Table I shows that the RNAse content of cells from the peritoneum is very much greater than that from the thymus and lymph nodes. The

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Table I. Ribonuclease activity of cell suspension from the peritoneum, thymus and lymph node cells of normal and cortisone-treated mice

Treatment of mice	RNAse[a] activity of cells[b] from		
	Peritoneum	Thymus	Lymph nodes
None	12.2 ± 2.1	0.56 ± 0.2	1.7 ± 0.5
1 day following cortisone[c]	99.0 ± 24.0	5.0 ± 2.9	2.1 ± 0.8
2 days following cortisone	102.0 ± 32.3	2.1 ± 0.6	3.8 ± 1.9
3 days following cortisone	26.6 ± 10.7	4.1 ± 2.2	3.4 ± 1.1

[a] Expressed as 10^{-15} g of crystalline pancreatic RNAse per cell. [b] Cells were pooled from 5 mice for each of the 3 experiments (mean of 3 separate experiments ± S.D.). [c] 5 mg cortisone acetate (Fredriksberg Chemical Laboratories Ltd., Copenhagen) given s.c.