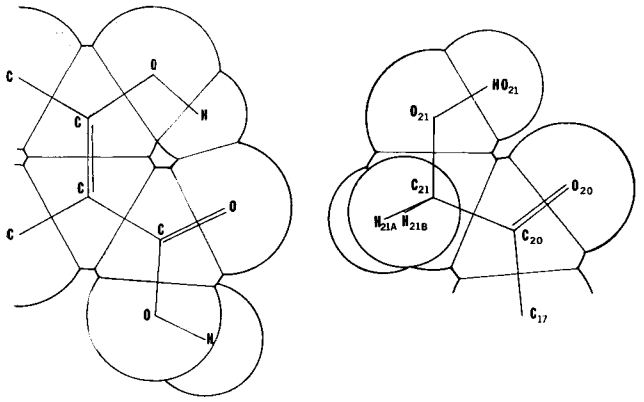

(4a) 4(b)

Figure 4—*Van der Waals outlines of* (a) *portion of salicylic acid, showing the presence of strong intramolecular hydrogen bonding;* (b) *the C-17 side chain of cortisone, showing the unfeasibility of intramolecular hydrogen bonding.*

lography (13), and intramolecular hydrogen bonding has been shown to exist. It is quite obvious that the additional atom in the hydrogen-bonding hexagon (Fig. 4*a*) has brought the atoms into the correct steric configuration for hydrogen bonding, but in cortisone (Fig. 4*b*) this cannot occur. The conclusion is that there is no intramolecular hydrogen bonding in the side chain of cortisone and that even in solution, the geometry of these atoms will not allow the necessary steric relationships to exist in order to effect it.

Intermolecular hydrogen bonding occurs in the cortisone crystals, between O-3 and the 17α-hydroxyl group of adjacent molecules, as shown in Fig. 3. There is a possibility of very weak hydrogen bonding involving O-3 and the C-21 hydroxyl group of an adjacent molecule, but the geometry of the "bond," as shown in Fig. 3, is at the limit of acceptable values.

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Action of Adenosine Triphosphate on the Depressed Spontaneous Electrical Activity of the Dog Cerebral Cortex

G. BENZI, E. ARRIGONI, A. FERRARA, and P. MASCHERPA

Abstract □ The repeated suppression of the ventilation and the circulation of the brain in the dog, induces a high depression or a silence of electrical activity of the cortical and subcortical centers. The recovery of respiratory and circulatory conditions produces only a partial spontaneous reversion, which is particularly improved by adenosine triphosphate, selectively perfused into the circle of Willis.

Keyphrases □ Adenosine triphosphate (ATP) activity—cerebral cortex □ Cortical electrical activity depression—ATP effect □ EEG recording—ATP cortical effect

Many investigators have been interested in the modification of the normal or pathological electroencephalogram (EEG) induced by the systemic or local administration of drugs to experimental animals or man. However, a number of workers agree that electrical activity of the cortex is greatly modified by changes in blood CO_2 and O_2. An increase in CO_2 is associated with a shift in energy toward the fast side of the spectrum with a decrease in the total amount of energy; on the contrary a low P_{CO_2} induces a change in energy toward the slow side of the spectrum with an increase in the total amount of energy. A decrease in amplitude with an increase in frequency, as it occurs with high CO_2 values, does not necessarily indicate that less potential energy is being used, but it may indicate that the EEG recording apparatus is less efficient at high frequencies (1). Nevertheless, during a condition of short asphyxia by suspension of artificial ventilation, an actual decrease or suppression of the cerebral electrical activity can be induced; restoring artificial ventilation results in a normal EEG (2). The acute and severe anoxia induced by inhalation of 100% nitrogen gave progressive

Table I.—Action of Natural Recovery of Respiratory and Circulatory Conditions, and of the Subsequent Treatment with ATP ($5.10^{-5}M$ in Saline Solution) Directly Perfused into the Circle of Willis (0.5 ml./min. for 30 min.) on the Depressed Spontaneous Electrical Activity of the Cerebral Cortex in the Dog[a]

Cardiac Arrest	No. of Dogs	Total Time of Cerebral Silence		EEG after 1 hr. of Natural Recovery				EEG after 1 hr. of Natural Recovery and 1 hr. of Perfusion with Saline Solution (0.5 ml./min. for 30 min.)				EEG after 1 hr. of Natural Recovery and 1 hr. of Treatment with ATP			
		Min.	Dogs	To Normal Condition	Depression Light	Depression Moderate	Depression Deep	To Normal Condition	Depression Light	Depression Moderate	Depression Deep	To Normal Condition	Depression Light	Depression Moderate	Depression Deep
No	23	<8	1	—	—	1	—	—	—	1	—				
			5	1	2	1	1					4	—	1	—
		8–16	4	1	2	1	—	1	3	—	—				
			7	1	1	3	2					3	2	1	1
		>16	2	—	—	1	1	—	—	1	1				
			4	—	1	—	3					1	1	1	1
Yes	21	<8	1	—	—	—	1	—	—	—	1				
			1	—	—	—	1					—	1	—	—
		8–16	3	—	1	—	2	—	—	1	2				
			6	—	1	1	4					1	3	1	1
		>16	5	—	1	2	2	—	—	3	2				
			5	—	1	1	3					1	2	1	1

[a] EEG pattern scale (related to basal values = 100): deep depression = reduction >70%; moderate depression = reduction from 40–70%; light depression = reduction from 10–40%. Statistical analysis of the difference between the effect of ATP perfusion and the effect of saline solution perfusion (*a*) in inducing an improvement of the EEG pattern: $\chi^2 = 10.34$ ($\chi_{0.01}^2 = 6.64$); (*b*) in inducing a return to normal condition: $\chi^2 = 3.94$ ($\chi_{0.05}^2 = 3.84$).

reduction of amplitude and slowing in the EEG, first in the theta, then in the delta range. Finally the recording became flat. When air was breathed after anoxia for 2 min., the EEG showed normal rhythm (3).

In the present research prolonged depression of the cortical electrical activity was produced by asphyxia, and the modifications caused by introducing adenosine triphosphate (ATP) directly into the circle of Willis of the dog were observed.

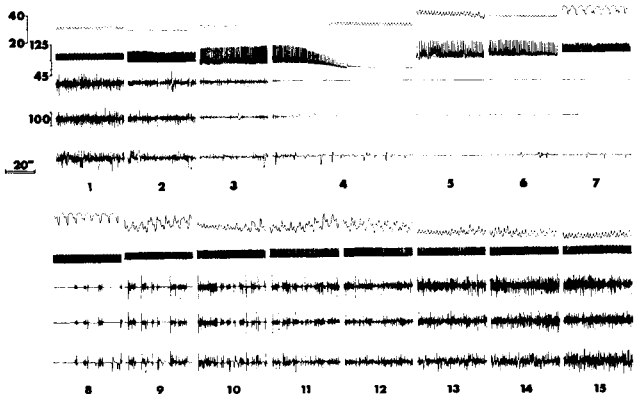

Figure 1—*Action of ATP on the depressed electrical activity of the dog's cerbral cortex. The record from top to bottom: pressure (cm. H_2O) in duodenal balloon; blood pressure (mm. Hg); frontal, parietal, occipital EEG patterns (μV.). 1 = basal conditions by anesthesia, after surgical procedure; between 1 and 2 = repeated suppression of the artificial ventilation with carotid arteries closure; 2 = 10 min. later; 3 = 20 min. later: 4 = 24 min. later (note the cardiac arrest): between 4 and 5 = 3 min. of cardiac arrest [the revival was accomplished by direct active myocardial massage and by intracardial injection of isoproterenol (50 mcg.); the carotid arteries were opened with the recovery of the artificial ventilation]; 5 = 5 min. later; 6 = 10 min. later: 7 = 20 min. later; 8 = 30 min. later; 9 = 60 min. later [the perfusion with ATP (5.10^{-5} M in saline solution) started]; 10 = 5 min. later; 11 = 10 min. later; 12 = 20 min. later; 13 = 30 min. later (the perfusion stopped); 14 = 60 min. later; 15 = 120 min. later.*

METHOD

The experiments were carried out on 48 dogs (9.6–17.8 kg.) preanesthetized with urethan (0.4 g./kg. i.p.). Anesthesia was induced and maintained in closed circuit by nitrous oxide, cyclopropane, or ethyl ether. The animals were given artificial ventilation after tracheal intubation by Warne tube, following succinylcholine chloride (1 mg./kg. i.v.) administration. The general condition of the animals was investigated by the evaluation of the systemic arterial blood pressure (from a cannula inserted into a femoral artery), and of the tone and motility of both the duodenum and small intestine (from a rubber balloon).

By drilling, monopolar electrodes were set in place in left and right frontal, parietal, and occipital areas; in 11 dogs, bipolar electrodes were set in place in thalamus and in cerebral peduncle. Arterial blood pressure, intestinal tone and motility, and cerebral electrical activity were recorded by a 12-channel polygraph (Physioscript EE 12 Schwarzer).

The operative procedure for arterial injection into the brain consists primarily of isolation of the common carotid arteries and of ligatures of all their branches, except the internal carotid arteries and the right thyroidea superior artery. The right external jugular vein was isolated, and the vertebral vessels were ligated before their entrance into the transverse foramen of C_2 or C_3. The numerous muscular branches arising from the vertebral vessels, the anastomosis between vertebral and carotid arteries, the muscular vessels of the neck, the vessels running under the carotid arteries and vagus nerves, the zygomatic, maxillary, auricolar, and supraorbital vessels were occluded by ligature or compression. The isolated thyroidea superior artery was cannulated by a polystan tube and connected with a perfusor (Palmer). Hematocrit, clotting-time, pH, and lactic acid were measured with blood samples from a polystan tube inserted into the isolated jugular vein; LDH was measured in cerebrospinal fluid. The basal electroencephalographic pattern by anesthesia, after the surgical procedure, was maintained at fourth of Faulconer's levels (4).

Depression of the cerebral electrical activity was produced during treatment with tubocurarine (0.1 mg./kg. every 30 min. i.v.) by suppression of artificial ventilation and by the subsequent (5–8 min.) closure of the carotid arteries. This condition induced a series of burst suppression patterns in the EEG with ultimate electrical silence; myocardial depression began, the animals were given artificial ventilation, and the carotid arteries were opened again. Spontaneous partial recovery occurred in both EEG and myocardial activity: after 5 to 15 min. artificial ventilation was suppressed and the carotid arteries closed again.

This procedure was repeated many times (4–8). In 21 experiments the last myocardial depression became extreme during 2 to 4 min., and revival was accomplished only by a direct, active myocardial massage and, in 11 dogs, also by injection of 25–50 mcg. of isoproterenol into an auricle rather than a ventricle.

After the last myocardial depression, with or without cardiac arrest, both artificial ventilation and carotideal cerebral circulation were given for 3 hr. During the second hour in 16 dogs saline solution was perfused into the circle of Willis at the rate of 0.5 ml./min. for the first 30 min., and in 28 dogs ATP (5.10^{-5} *M* in saline solution) was perfused into the circle of Willis at the rate of 0.5 ml./min. for the first 30 min.

RESULTS AND DISCUSSION

The repeated suppression of artificial ventilation, with carotid arteries closure, induced a high depression or silence in the EEG, with only partial spontaneous reversion after recovery of respiratory and circulatory conditions (Table I) during the time of observation. Parallel behavior was shown by the subcortical centers. The subsequent treatment with ATP, selectively perfused into the circle of Willis, improved the partial spontaneous reversion of the EEG depression, as summarized in Table I and exemplified in Fig. 1, which shows one of the best results obtained.

It is possible to note also (*a*) the lesser natural recovery in EEG pattern induced by the cardiac arrest; (*b*) the inverse correlation between the time of cerebral silence and the recovery of the EEG pattern, with or without ATP treatment; and (*c*) the lack of EEG modification by intracardiacal injection of isoproterenol during the direct active myocardial massage after cardiac arrest.

During the cerebral asphyxia, with related depression of electrical activity of the cortex, the blood samples from the jugular vein showed no modification of hematocrit and clotting-time, while the pH decreased and lactic acid increased. A rise of LDH was detectable in the cerebrospinal fluid. The recovery of respiratory and circulatory conditions corrected partially the modified values, with a possible return to normal conditions after treatment by ATP.

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Light-Scattering Investigation of Protamine Sulfate

K. C. YEH*, S. P. LOUCAS, and H. M. HADDAD

Abstract □ A study of the weight average molecular weight of protamine sulfate using light-scattering photometry at 436 mμ was undertaken. Results obtained indicate a molecular weight of 3600 for the free base. Dissymmetry and second virial coefficient data are discussed relative to solute-solute interactions.

Keyphrases □ Protamine sulfate—molecular weight determination □ Light-scattering spectrophotometry—analysis □ Refractive index increment—protamine sulfate solution

Protamine sulfate, a heparin antagonist, is obtained from the sperm or mature testes of fish (1) belonging to the genus *Oncorhynchus* Suckley, Salmo Linne, or Trutta Jordan et Evermann (Fam. *Salmonidae*). Since it is derived from animal sources, previous molecular weight determinations have led to the reporting of conflicting results. From end-group determinations, Phillips (2) assigned a molecular weight of 3800. Unpublished data by Callanan (3) attributed a maximal average molecular weight of 5000 to salmine on the basis of particle weight distribution.

Since the literature is void of experimental details, the discrepancy between number and weight average molecular weights caused further study of the light-scattering patterns of protamine sulfate solutions.

EXPERIMENTAL

Material—Samples of protamine sulfate powder were used (lots ONPO2E and ONPO2H, obtained from Eli Lilly Laboratories). Sulfate and nitrogen elemental analysis (4), on an anhydrous basis, gave a 17.7 and 23.3% content, respectively. Triple-distilled sterile water was used as solvent for all solutions with an apparent optical turbidity of 10^{-5} cm.$^{-1}$. All solution concentrations were calculated on the dried basis.

Light-Scattering—Refractive index increments (dn/dc) and weight average molecular weight (Mw), defined as $(n_{soln.} - n_{soln.})/c$ and the ratio $\Sigma NiMi^2/\Sigma Ni\Sigma Mi$, respectively, were obtained with a differential refractometer (Brice Phoenix) and modified dual photomultiplier type photometer (models 2000), using incident unpolarized light of 4358 Å. All samples measured gave a dn/dc of 0.180 ± 0.001 ml./g. suggesting minimum sample heterogeneity.

Temperature control was achieved by circulating thermostated water at 25 ± 0.1° through a cored light scattering cell table and jacket.

Scattering intensity was measured at 90° to the incident beam (I_{90}) relative to transmitted light (I_0) at 0°. Apparent turbidity t, and dissymmetry z, defined as the angular ratio i_{45}/i_{135} were carried out with a 40 × 40-mm. semioctagonal cell.

Light absorption at 436 mμ, depolarization, and fluorescence measurements were negligible with transmittance being greater than the limiting value of 63%.

The effect of pH on scattering was examined for solutions containing 12 × 10^{-3} g./ml. protamine sulfate in phosphate buffer, $\Gamma/2 = 0.1$, pH = 6.9–9.5. Data for all solutions were identical suggesting the absence of pH effects in this range.