EFFECT OF CHRONOTROPIC DRUGS ON OSCILLATORY ACTIVITY IN THE SINO-ATRIAL NODE. Theodore C. West. Department of Pharmacology, University of Washington School of Medicine, Seattle 5, Washington.

A portion of rabbit atrium connected to the rate-dominating region of the sino-atrial node was mounted for the recording of transmembrane potentials and contraction force. A modified Ringers solution containing 2.7 mM/L of potassium was used. The experiments were conducted at 37°C. The external sodium concentration was reduced to 26 per cent of normal. At this concentration spontaneous discharge from the sino-atrial node usually ceased, whereupon electrical stimulation of the atrium caused a propagated impulse to spread into the node and produce action potentials. Subthreshold, damped oscillations were observed in the node following these single, induced spikes. The administration of catecholamines increased the amplitude and the frequency of the oscillations. Choline esters exerted the opposite effect. From the equations for the frequency and damping of linear oscillations, the damping resistance and the inductance were calculated from suitable experimental data. The qualitative effects of positive and negative chronotropic drugs on the frequency of pacemaker discharge were shown to be independent of the concentration of external sodium.

LARGE SCALE PREPARATION AND PURIFICATION OF SUBSTANCE A. C.G. Huggins and E.J.Walaszek. Dept. of Pharmacology, Univ. of Kansas Medical School, Kansas City, Kansas.

Efforts to adequately characterize the polypeptide, substance A, have been hampered by lack of sufficient purified material. The present report is a result of work directed toward the preparation of a relatively large amount of dry, stable powder. Sixteen liters of 1% α-amylase and 16 liters of 1% fraction IV-4 of human plasma protein were mixed and incubated for 1¼ hours at 4°. All subsequent manipulations were carried out at 4°. Deactivated charcoal (1.5 gm/100 ml) was added to adsorb the polypeptide and then separated by centrifugation. The charcoal was washed successively, with 5 l of normal saline, distilled water and absolute ethyl alcohol. The active material was eluted from the charcoal with glacial acetic acid, evaporated under reduced pressure at 40°C and clarified by high speed centrifugation. The active material was precipitated by addition of 9 vol. of anhydrous ethyl ether. Further purification was obtained by redissolving the precipitate in glacial acetic acid, filtering through a bacteriological filter and precipitation with ethyl ether. Yield: 20 g of a light tan powder (80% of theoretical). Material of a higher degree of purity was obtained after 2 successive column chromatographic separations using gradient elution (ammonium acetate buffer, 0.02M pH 6.0 to 0.2M pH 7.0) from a carboxymethyl cellulose column. Studies concerning the electrophoretic mobility and amino acid composition of the purified polypeptide will be reported.

LUNG COMPLIANCE MEASUREMENT OF EXPERIMENTAL PULMONARY EDEMA COMPARED WITH POST-MORTEM CRITERIA. R.H.Rech*# and H.L.Borison. Dept. of Pharmacology, U. of Utah Coll. Med., Salt Lake City.

Acute pulmonary edema was induced by bilateral cervical vagotomy in guinea pigs weighing from 300 to 400 g. The most useful means of quantifying extent of edema has been lung-to-body weight ratio. This measurement was compared with lung specific gravity (S.G.) determined by immersion in silicones of varying densities. Non-edematous lungs floated in silicone of 0.76 S.G. while lungs with marked edema required a silicone of 0.94 S.G. for buoyancy. The relationship of S.G. to weight ratio is complicated by amount of trapped air. This factor was accounted for by measuring lung volume. It was desired also to follow progression of lung edema in situ. Thoracic compliance (V/P) was determined by means of intermittent positive pressure ventilation with various volumes (2,4,6 and 8 ml) delivered at different frequencies (0,46,72,110 and 150 cycles/min). Values at low frequencies served as a measure of lung elasticity while those at high frequencies reflected resistance to air flow. Development of edema was accompanied by a decrease in compliance, especially at volumes of 4 to 6 ml. The decrease was proportionately greater at lower frequencies, thus indicating a reduction in lung elasticity due to edema formation. (Supported by USPHS Grant B-491) #NIH Postdoct. Fellow BF-9406.

MEASUREMENT OF ADENOSINE-3',5'-PHOSPHATE (CYCLIC 3,5-AMP) ACTIVITY IN HEART, OTHER TISSUES, AND URINE. R. W. Butcher,* Earl W. Sutherland, and T. W. Rall. Dept. of Pharmacology, Western Reserve University School of Medicine, Cleveland, O.

Present estimates of concentrations of cyclic 3,5-AMP in mammalian heart ($1.0\text{-}3.0 \times 10^{-7}$ M/kg) and liver (2×10^{-7} – 2×10^{-6} M/kg) are based on an assay involving the activation of phosphorylase. A purified phosphodiesterase from heart was used to inactivate the cyclic 3,5-AMP, providing additional specificity. These estimates are inexact, because inhibitors from tissues, especially heart, influence the assay. It was found that glucose-1-phosphate and glucose-6-phosphate inhibit the assay in low concentrations (10^{-4} M), while ADP inhibits at somewhat higher concentrations. It appears that enzymatic digestion of heart extracts with potato apyrase, etc., followed by ion exchange procedures, increases the accuracy of the estimation. A material that appears to be similar to or identical with cyclic 3,5-AMP was found in the urine of dog and man. In man, values from 2-7 μmoles per day were found. (Supported by U. S. Public Health Service grants Nos. H-2745 and HTS-5228 and a grant-in-aid from The Eli Lilly Research Laboratories.)

REFLEX CARDIOVASCULAR AND RESPIRATORY RESPONSES TO SODIUM SALICYLATE IN THE CAT. R. Rosenstein*# and H. L. Borison. Dept. of Pharmacology, U. of Utah Coll. Med., Salt Lake City.

Rapid intravenous injection of sodium salicylate (100-200 mg/kg) produced an immediate but short-lasting reaction which included (1) respiratory apnea, followed by tachypnea, (2) bradycardia, and (3) a profound fall in blood pressure. Tachyphylaxis was not observed following repeated administration. Atropine (1 mg/kg) eliminated the bradycardia but only slightly diminished the hypotensive response. Vagotomy eliminated the respiratory and bradycrotic effects of salicylate and greatly reduced the hypotensive response. Subsequent denervation of the carotid bifurcation further reduced the hypotensive response. Carotid denervation prior to vagotomy did not detectably alter the total reaction to salicylate. Injection of salicylate into the left atrium evoked a cardiovascular pattern similar to that obtained following intravenous administration. On the other hand, the respiratory response was less intense, thus indicating that receptors in the pulmonary circuit as well as in the distribution from the left side of the heart are concerned in the reflex reaction to intravenous salicylate. Approximately 2.5 times more sodium chloride than sodium salicylate, at the same osmolarity (3.1M), were required to evoke a comparable reflex effect. It appears that the reflex response to sodium salicylate is not due to hyperosmolarity of solution. (Supported by USPHS Grant B-491) #NIH Predoct. Fellow BF-7870.

ANTAGONISTS OF THE PHARMACOLOGICALLY ACTIVE POLYPEPTIDE SUBSTANCE A. E.J.Walaszek, C.G.Huggins and C.M.Smith*. Dept. of Pharmacology, Univ. of Kansas Medical School, Kansas City, Kan.

We have reported previously that the hypertensive polypeptide substance A obtained by incubating fraction IV-4 of human plasma protein with α-amylase was similar to angiotensin II isoleucine^5 (octapeptide). This was ascertained by means of parallel assays and chromatographic analysis. In this report we shall discuss various antagonists to the stimulatory action of substance A on the guinea pig ileum. The pharmacologically active polypeptides bradykinin, substance P and various synthetic angiotensins (decapeptides and octapeptides) were also studied. Atropine (10^{-6}) had an antagonistic effect on the actions of substance A, substance P and the angiotensins but it had no effect on the action of bradykinin. Antihistaminics did not influence the contractions produced by these polypeptides. Lysergic acid diethylamide (LSD-25) potentiated the effects of substance A, substance P and bradykinin. 2-Brom-D-lysergic acid diethylamide (BOL-148) diminished the effect of substance P but did not influence the effects of bradykinin and substance A. Morphine (10^{-6}) had a profound action on the stimulatory effects of substance A and the angiotensins but no action against bradykinin and only a minimal action against substance P. Phenoxybenzamine (10^{-7}) was an antagonist of substance A and the angiotensins but not of bradykinin.