a return of these substances to or above normal levels, have been repeatedly described as characteristic of the adrenal response to stress. The alterations in cholesterol, ascorbic acid and stainable lipids have been considered by Sayers and Sayers[16] to closely parallel each other. Indeed the observation by these workers of a simultaneous decrease in cholesterol and sudanophilic material has prompted them to identify the latter with cholesterol esters. The present study shows that it is possible to dissociate the sudanophilia from the cholesterol content of the adrenal cortex. LAP, either alone or in conjunction with stilbestrol, decreases the cholesterol but increases the sudanophilia of the adrenal. This suggests that the sudanophilic substance of the cortex is probably not cholesterol. Earlier experiments[17] have demonstrated that, under certain conditions, purified ACTH can cause a similar dissociation. Finally it appears from the above data that LAP, given with stilbestrol, markedly inhibits that loss of sudanophilic granules from the cortex which is characteristic of stilbestrol treatment.

[13] Torrance, C. C., *J. Biol. Chem.*, 1940, **132**, 575.

[14] Harkins, H. N., and Long, C. N. H., *Am. J. Physiol.*, 1945, **144**, 661.

[15] Sayers, G., and Sayers, M. A., Tsan-Ying Liang, and Long, C. N. H., *Endocrinology*, 1945, **37**, 96.

[16] Sayers, G., and Sayers, M. A., *Rec. Progr. in Horm. Res., Proc. Laur. Horm. Conf.*, 1948, **2**, 81.

[17] Constantinides, P., Fortier, C., Skelton, F. R., Timiras, P., and Selye, H., in press.

Further experiments are under way to study the effects of purified corticotrophic preparations on the adrenal changes elicited by nonspecific stress.

Summary. The adrenal cholesterol and ascorbic acid levels were studied in relation to the stainable lipids of the gland in rats treated with a lyophilized anterior pituitary preparation (LAP), given alone or in conjunction with a synthetic folliculoid (stilbestrol):

1. In stilbestrol treated rats, an almost total depletion of stainable lipids was observed in conjunction with low cholesterol and ascorbic acid values.

2. LAP caused accumulation of sudanophilic lipids in the adrenal cortex but significantly lowered the cholesterol and ascorbic acid levels.

3. A similar dissociation of sudanophilic material from cholesterol and ascorbic acid, was even more evident when LAP was administered concurrently with stilbestrol. The effect of stilbestrol on sudanophilia was counteracted by LAP.

4. These observations show that the corticotrophic effect of folliculoids cannot be solely ascribed to the liberation of anterior pituitary corticotrophin such as is present in LAP.

This investigation was supported by a research grant from the National Heart Institute, U. S. Public Health Service. The authors gratefully acknowledge the histological assistance of Dr. P. S. Timiras and the technical help of K. Nielsen and M. Parent.

Received April 19, 1949. P.S.E.B.M., 1949, **71**.

17104. Effect of Various Drugs on Epinephrine-Induced Pulmonary Edema in Rabbits.*

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Large doses of epinephrine are known to cause a fulminating pulmonary edema in a number of species of animals.[1-3] Various

* Parke, Davis and Co., Detroit, Mich., supplied a grant in support of this investigation, as well as the adrenergic blocking drugs employed.

[1] Meltzer, S. J., *Am. Med.*, 1904, **8**, 191.

[2] Emerson, H., *Arch. Int. Med.*, 1909, **3**, 368.

[3] Bovet, D., and Bovet-Nitti, F., *Structure et activité pharmacodynamique des médicaments du système nerveux végétatif*, S. Karger, Bale, 1948, p. 23.

workers have claimed that atropine,[4,5] hypnotics,[6] tissue extracts,[7] antihistaminics[8] and pituitrin[9] can diminish or prevent this edema formation. Furthermore, various adrenergic blocking drugs, as well as vasodilator drugs have been shown to be capable of reducing the mortality which follows toxic doses of epinephrine in mice[10] and rats.[11,12] A variety of explanations concerning the cause of pulmonary edema have been based, in part, on data relating to the effects of drugs on the toxicity of epinephrine.

The drugs which have been reported to protect against epinephrine-induced pulmonary edema vary widely in properties and several are relatively specific in action. If the claims made are true it would suggest that numerous mechanisms are involved in edema production or that the several protective drugs have a common property, which seems quite unlikely. Preliminary to studies aimed more directly at the elucidation of the mechanisms involved in the production of pulmonary edema and the control by drugs it was considered advisable to re-examine the effects of some of these drugs on epinephrine-induced pulmonary edema under standardized experimental conditions.

Method. Drugs were administered as indicated in Table I to male rabbits weighing between 2 and 3 kg prior to the intravenous injection, in 15 seconds, of 0.375 mg/kg of epinephrine (calculated as the base). The epinephrine solution employed (0.1%) was

TABLE I. Alteration of Pulmonary Edema and Mortality Due to Large Doses of Epinephrine in Rabbits.

	No. exp.	Mean body wt, kg	Mean lung wt, g ± s	Diff. from epinephrine controls, g ± s	P*	% mortality†	Diff. from epinephrine controls	P*
Control Groups.								
Epinephrine	18	2.32	21.77 ± 7.19	—	—	61.1	—	—
Normal lung wt	12	2.59	10.58 ± 1.41	—	—	—	—	—
Pretreated Groups‡								
Pentobarbital Na, 25 mg/kg I.V.	6	2.52	22.34 ± 6.36	+ 0.57 ± 3.3	>0.5	66	+ 4.9	>0.5
Phenobarbital ,, 50 ,, S.C.	6	2.60	34.95 ± 14.49	+13.18 ± 4.31	<0.01	80.8	+28.9	>0.1, <0.2
Neoantergan maleate, 2.5 mg/kg I.V.	9	2.33	19.44 ± 5.99	— 2.33 ± 2.80	>0.4	55.6	— 5.5	>0.5
Phenergan HCl, 1.5 mg/kg I.V.	7	2.74	26.21 ± 10.84	+ 4.44 ± 3.59	>0.2	57.1	— 4.0	>0.5
,, ,, 20 ,, S.C.	8	2.69	24.53 ± 6.05	+ 2.76 ± 2.90	>0.3	12.5	—48.6	<0.01
Atropine sulfate, 5 mg/kg I.V.	9	2.37	14.95 ± 6.31	— 6.83 ± 2.44	>0.02, <0.05	33.0	—28.1	>0.1, <0.2
SY-28,§ 0.25 mg/kg I.V.	5	2.40	9.13 ± 0.66	—12.64 ± 3.27	<0.01	0	—61.1	<0.01
SY-2,‖ 2.5 mg/kg I.V.	6	2.36	10.83 ± 2.31	—10.94 ± 3.02	<0.01	0	—61.1	<0.01

* Statistically significant when 0.05 or less.
† Mortality figures are those based on the numbers of animals dying spontaneously during 15-minute observation period following epinephrine.
‡ Intravenous pretreatment was instituted 10 minutes before epinephrine. Drugs administered subcutaneously were given 30 minutes before the standard intravenous dose of epinephrine of 0.375 mg/kg (as the base).
§ N-(2-bromoethyl)-N-ethyl-1-naphthalenemethylamine • HBr. ‖ N-(2-chloroethyl)-N-ethylbenzhydrylamine • HCl.

[4] Auer, J., and Gates, F. L., *J. Exp. Med.*, 1917, **26**, 201.

[5] Bariety, M., and Kohler, D., *Compt. rend. Soc. de biol.*, 1948, **142**, 280.

[6] Luisada, A., *Arch. f. Exp. Path.*, 1928, **132**, 313.

[7] Boivin, G., *Compt. rend. Soc. de biol.*, 1929, **101**, 251.

[8] Halpern, B. N., Hamburger, J., and Cruchand, S., *Acta allergologica*, 1948, **1**, 97.

[9] Elkeles, A., *Brit. J. Radiol.*, 1948, **21**, 472.

[10] Loew, E. R., and Micetich, A., *J. Pharm. and Exp. Therap.*, 1948, **93**, 434.

[11] Nickerson, M., and Goodman, L. S., *J. Pharm. and Exp. Therap.*, 1947, **89**, 167.

[12] Rothlin, E., *Bull. de l'Acad. Suisse des Sci. Med.*, 1946-47, **2**, 1.

made by dissolving the base in saline with the addition of a few drops of HCl. Concentrations of other drugs in saline were adjusted so that a given dose was administered in 0.5 ml/kg. Control data were obtained concurrently from animals receiving only epinephrine or no treatment whatsoever. Following the intravenous injection of epinephrine, the rabbits were observed for 15 minutes, at which time, if surviving, they were sacrificed by a sharp blow on the head. All lungs were removed 10 minutes after death, freed of adhering tissue, blotted on absorbent paper and wet-weighed. The indication of effectively altering the toxic response to epinephrine was the presence of a statistically significant reduction in lung weight and/or mortality from that of the controls receiving only epinephrine (Table I).

Results and discussion. The lungs of epinephrine-treated rabbits were markedly hemorrhagic and edematous and weighed double that of normal lungs. A frothy fluid, usually uncolored but sometimes salmon colored, frequently completely filled the trachea and bronchi and appeared to account for the respiratory embarrassment, cyanosis and asphyxial convulsions which preceded death. Fluid frequently escaped from the nostrils and mouth during asphyxial convulsions or when survivors were killed 15 minutes after injection of epinephrine. This fulminating, severe pulmonary edema was probably the main cause of death which occurred in 3 to 15 minutes in 61% of the 18 control animals. Pretreatment with a variety of drugs was instituted in attempts to prevent the edema. During each daily experiment several control rabbits and several rabbits treated with each drug were included, thus yielding data which could be pooled and compared (Table I).

Barbiturates. Luisada[6] reported that hypnotics in general were capable of reducing the mortality and pulmonary edema induced with epinephrine in rabbits. He believed that such drugs protected by suppressing a central component of a contributory pulmonary vascular reflex which was brought about by epinephrine or the resultant severe hypertension.[6] The results herein reported with pentobarbital in a near anesthetic dose and phenobarbital in a sedative dose do not support such a contention. Neither of these barbiturates exerted any protective action under conditions which appear to be essentially identical to those described by Luisada. Phenobarbital actually caused a significant increase in lung weight and the data suggest an increased mortality.

Antihistaminics. Experiments conducted by Staub[13] indicate that epinephrine may cause the release of significant amounts of histamine in the body. If the toxic effects of large doses of epinephrine in rabbits are accentuated by histamine released from tissues, it would be expected that pretreatment with antihistaminics would confer protection.

The treatment with Pyranisamine (Neoantergan maleate†), a potent and specific antihistaminic did not prevent the increase of lung weight or reduce mortality due to epinephrine (Table I). N-β-Dimethylaminopropylthiodiphenylamine.HCl (Phenergan, 3277 RP)† administered intravenously likewise afforded no protection, although larger doses injected subcutaneously reduced mortality but did not decrease pulmonary edema. This suggests that death in rabbits due to epinephrine is not attributable solely to pulmonary edema, or that undetected decreases in degree of edema were sufficient to reduce mortality.

These data do not confirm the results reported by Halpern *et al.*[8] who found that the same large doses of Phenergan under essentially the same experimental conditions completely protected rabbits from the toxic effects of epinephrine. Reuse[14] using the same doses of Phenergan in guinea pigs, reported that this drug reduced mortality due to epinephrine, although the protection conferred by Phenergan was not complete. He states that immediately after epinephrine, the treated animals showed signs of respiratory embarrassment. While this suggests the presence of congestion and edema, no routine examination of the lungs was made. However, the lung weights of 3 such animals were somewhat above nor-

[13] Staub, H., *Experientia*, 1946, **2**, 29.

† Supplied through the courtesy of Merck and Co., Rahway, N. J.

[14] Reuse, J. J., *Compt. rend. Soc. de biol.*, 1948, **142**, 638.

mal, even at 2 hours after epinephrine.

Atropine. Various authors[4,5] have claimed that atropine protects against epinephrine-induced pulmonary edema. Phenolic ethers of amino alcohols, possessing atropine-like properties as well as antihistamine and epinephrine blocking properties, are said to protect.[5] On the other hand, significant protection by atropine has been denied.[6,15]

Table I shows that a large intravenous injection of atropine sulfate partially prevented to a significant degree the increase of lung weight. Mortality was not significantly decreased probably because of the small number of animals employed. However, doses less than 5.0 mg/kg did not protect, and the dose necessary to confer protection was relatively large considering the potency of the agent. In addition, the protective effect of atropine is slight when compared to that produced by the adrenergic blocking agents.

Adrenergic blocking drugs. It has been shown that N-(2-bromoethyl)-N-ethyl-l-naphthalenemethylamine.HBr (SY-28) is a potent adrenergic blocking agent,[16,17] as well as one of the most potent histamine antagonists known.[16] A closely related compound, N-(2-chloroethyl)-N-ethylbenzhydrylamine.HCl (SY-2) is a specific adrenergic blocking agent.[18] Both of these compounds, in comparatively small doses, prevented death and pulmonary edema due to epinephrine in rabbits, as is shown in Table I. It is emphasized that these were the only drugs studied which completely protected the animals against the toxic effects of epinephrine.

Other adrenergic blocking agents are capable of reducing epinephrine-induced pulmonary edema. Rothlin[15] recently reported that ergotamine had such properties. Dibenamine and various other derivatives of β-chloroethylamine, benzyl-imidazoline (Priscol) and yohimbine, when given orally, reduced the toxicity of epinephrine injected intraperitoneally in mice.[10] Under these conditions, the dioxanes, 883F and 933F, were ineffective following moderate doses in mice.[10] Presumably the protective action of most adrenergic blocking drugs in mice is due to the prevention of pulmonary edema since increased lung weights were readily demonstrable after epinephrine injection.[19]

We believe the data obtained from rabbits and herein presented and the findings reported for mice,[10] rats[11,12,15] and rabbits,[6] reveal clearly that pulmonary edema and mortality following epinephrine administration are most effectively reduced by vasodilator drugs which are physiological antagonists of epinephrine and by adrenergic blocking drugs which pharmacologically block excitatory actions of epinephrine such as vasoconstriction and the resultant hypertension.

A more direct examination of the acute hypertensive action of epinephrine, and of the pressures within pulmonary vessels and of the effect of drugs thereon would appear to be the most promising approach to elucidation of factors involved in epinephrine-induced pulmonary edema. Hamilton, Woodbury, and Vogt[20] recorded pressure changes in the pulmonary artery and vein of the unanesthetized dog after large, but non-fatal, doses of epinephrine and observed a rise of pressure in both. They concluded that the increased pressures were due to failure of the left ventricle as a result of the high arterial pressure. Furthermore, direct observations of the heart by Auer and Gates,[4] Johnson,[21] and Barach, *et al.*[22] indicate that the left ventricle fails in the rabbit as well. This suggests that a high pulmonary circulatory pressure prevails after toxic doses of epinephrine and could account for the rapid transudation of fluid into the lungs.

Summary. 1. Pulmonary edema and mortality induced in rabbits by intravenous injections

15 Rothlin, E., *Report before Eleventh International Congress of Military Medicine and Pharmacy,* Basle, June 2-7, 1947.

16 Loew, E. R., and Micetich, A., *J. Pharm. and Exp. Therap.,* 1948, **94**, 339.

17 Stone, C. A., and Loew, E. R., *J. Pharm. and Exp. Therap.,* 1948, **94**, 350.

18 Loew, E. R., and Micetich, A., *Fed. Proc.,* 1947, **6**, 351.

19 Unpublished observations.

20 Hamilton, W. F., Woodbury, R. A., and Vogt, E., *Am. J. Physiol.,* 1938, **125**, 130.

21 Johnson, S., PROC. SOC. EXP. BIOL. AND MED., 1927-28, **25**, 181.

22 Barach, A. L., Martin, J., and Eckman, M., *Ann. Int. Med.,* 1938, **12**, 754.

of epinephrine were completely prevented by comparatively small doses of adrenergic blocking drugs, N-(2-bromoethyl)-N-ethyl-l-naphthalenemethylamine.HBr (SY-28) and N-(2-chloroethyl) - N - ethylbenzhydrylamine.HCl (SY-2).

2. It would appear unlikely that epinephrine toxicity and the pulmonary edema involved are importantly related to histamine release from tissues since the antihistaminics, Pyranisamine (Neoantergan maleate) and Phenergan (3277 RP), failed to prevent edema although large subcutaneous doses of Phenergan reduced mortality.

3. The barbiturates, pentobarbital sodium and phenobarbital sodium did not decrease mortality or edema.

4. The available evidence indicates that epinephrine toxicity is most readily reduced by vasodilator drugs which represent physiological antagonists, and by adrenergic blocking drugs which are pharmacological antagonists of epinephrine.

Received April 19, 1949. P.S.E.B.M., 1949, **71**.

17105. Myelination of the Hypothalamus and Its Relation to Thermoregulation in the Hamster.*

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Correlation of the ability to regulate body temperature in a cold environment with progressive myelination of the hypothalamus has been previously demonstrated in albino rats.[1] The ability of rats to maintain body temperature is acquired at ages ranging between 18 and 30 days. The earlier literature indicating that function of fiber tracts in the central nervous system may be dependent upon myelination of their component nerve fibers was briefly reviewed in a previous report.[1]

In the present report the results of an investigation of myelination in the hypothalamus of the hamster and the development of its ability to regulate against cold are presented. The investigation seemed indicated in view of the fact that hamsters are more immature at birth than rats.

Methods and materials. Six litters of Syrian hamsters were used in the present study. Their body temperatures were recorded by means of a Brown electronic continuous recording potentiometer and copper-constantan thermopiles. The thermopiles were inserted into the colons of the animals to a depth of 4-5 cm. Animals varying from 6-71 days of age were subjected to environmental temperatures of 5-8°C for periods varying from 30 minutes to 20 hours. After exposure the hamsters were anesthetized and perfused with 3½% potassium dichromate followed by Regaud's solution. The brains were then removed, divided in the median sagittal plane and placed in 5% potassium dichromate where they remained for 10 days days at a temperature of 37-39°C. After washing, dehydration, embedding, and sectioning at 20 μ they were stained according to the method of Ulett, Dow, and Larsell.[2] Sections were made in the parasagittal plane to permit study of all areas of the hypothalamus (rostral, caudal and middle) in each section. Representative sections from the lateral hypothalami of all members of a given litter were stained en masse and myelin was quantitated by means of a Photovolt electronic photometer as previously described.[1] In only two instances, because of the difficulty in procuring large litters of hamsters, was a litter-mate control sacrificed with the experimental animals.

* Supported by a grant from the Office of Naval Research.

1 Buchanan, A. R., and Hill, R. M., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 602.

2 Ulett, G., Dow, R. S., and Larsell, O., *J. Comp. Neur.*, 1944, **80**, 1.