lantoic fluid containing virus antigen. Another advantage in using hamsters is the ability of these animals to produce antibodies in response to low concentrations of virus. This makes it possible to prepare antisera to viruses in first or second egg passage. This is not always feasible with chickens because more virus is required to induce antibody formation in these animals. The value of hamster sera is slightly affected by a shortcoming; the hemagglutination-inhibition test is difficult to read because of the rapid settling of cells, giving rise to fuzzy or serrated discs of agglutinated cells.

Summary. Antisera prepared in hamsters by the intranasal instillation of allantoic fluids containing influenza virus were type-specific when used in the complement-fixation test with the soluble antigens; group- or subtype-specific (*e.g.* A prime) when used in the complement fixation test with the allantoic fluid antigen, and strain-specific in hemagglutination-inhibition tests.

It appears that the hamster is an animal best suited for quick identification of influenza viruses. It is suggested that their sera be used for systematic classification of strains.

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Effect of Epinephrine, Antihistaminics and Adrenergic Blockade on Egg White Edema in Adrenal Insufficient Rats.* (17484)

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Recently we reported a marked protection by 0.1 mg/kg epinephrine hydrochloride against the acute edema induced by the intraperitoneal injection of egg white in rats.[1] Selye[2] and Leger and Masson[3] showed that adrenalectomy greatly sensitized rats to this edema and led to death in a large percentage of the animals. Adrenal cortical extract protected such animals from dying *but not from the edema,* nor did it confer protection to animals with intact adrenals. Kellaway,[4] Wyman,[5] and Perla and Marmorston-Gottesman,[6] Wyman[7] and Ingle, *et al.*[8,9] showed that epinephrine antagonized histamine toxicity in adrenalectomized rats. The egg white edema syndrome could conceivably be caused by the local release of histamine or a histamine-like substance, since antihistaminics effectively combat the edema, as demonstrated by Leger and Masson,[10] Brown and Werner,[11] and ourselves.[1] The doses of antihistaminics required are considerable, however, which may also suggest that the protection results from precapillary arteriolar vasoconstriction[12] and decreased capillary filtration rather than "anti-

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1 Clark, W. G., and MacKay, E. M., PROC. SOC. EXP. BIOL. AND MED., 1949, **71**, 86.

2 Selye, H., *Endocrinol.*, 1937, **21**, 169.

3 Leger, J., and Masson, G. M. C., *Ann. Allergy*, 1949, **6**, 131.

4 Kellaway, C. H., *J. Physiol.*, 1923, **57**, 82.

5 Wyman, L. C., *Am. J. Physiol.*, 1928-29, **87**, 29.

6 Perla, D., and Marmorston-Gottesman, J., *Am. J. Physiol.*, 1929, **89**, 152.

7 Wyman, L. C., *Am. J. Physiol.*, 1929, **89**, 356.

8 Ingle, D. J., *Am. J. Physiol.*, 1937, **118**, 57.

9 Ingle, D. J., Nezamis, J. E., and Kuizenga, M. H., *Exp. Med. and Surg.*, 1947, **5**, 379.

10 Leger, J., and Masson, G., *Am. J. Med. Sci.*, 1947, **214**, 305.

11 Brown, B. B., and Werner, H. W., *J. Lab. Clin. Med.*, 1948, **33**, 325.

12 Haley, T. J., and Harris, H., *J. Pharm. Exp. Therap.*, 1949, **95**, 293.

TABLE I. Exp. 1.

Group	% incidence of edema	Degree of edema	% dead	Symptoms
1. Controls	100	3.5+	0	—
2. Adx untreated	80	3.5	10	30% cyanotic and all prostrated
3. Adx + epinephrine	0	0	0	None
4. Adrenal demedullated	80	4.0	0	"

TABLE II. Exp. 2.

Group	% incidence of edema	Degree of edema	% dead	Symptoms
1. Untreated controls	80	4	0	—
2. Adx untreated	100	4	100	Died in 1½-3 hr
3. Adx + epinephrine	50	3	0	Good condition
4. Adrenal demedullated (untreated)	75	4	0	—

histaminic action." Selye[2] and Leger and Masson[3] found that among other things, "alarming stimuli" or stress such as exercise, cold, India ink and formalin injections inhibited the reaction. Since epinephrine protects against egg white edema in the normal rat and against the enhanced histamine sensitivity in the adrenalectomized rat, it seemed possible that it would protect against the increased egg white sensitivity and mortality in adrenalectomized rats as effectively or more so than adrenal cortical extract. Since it was considered possible that "alarming stimuli" may act by releasing endogenous epinephrine, additional experiments were done in adrenal demedullated rats and rats in which adrenergic blockade was induced.

Methods and Results. The methods of producing and grading the severity of the edematous symptoms have been described elsewhere.[1] Two to 4 hours after the intraperitoneal injection of fresh, filtered (or redissolved lyophilized) egg white, the edema of the face and extremities was graded by the per cent incidence and by the 0–4+ degree of edema. In addition, where adrenal insufficiency was included, the per cent mortality and symptoms were recorded. Leger and Masson's observations of enhanced sensitivity following adrenalectomy were confirmed. Following adrenalectomy, the rats were kept on the stock diet (Purina Chow) and 1% NaCl + 0.2% $NaHCO_3$ drinking fluid for some time before they were given egg white. If the salt therapy was continued up to and following the injection, there were fewer deaths than if they were deprived of salt for 24 hours, but in any case the symptoms were much more severe than in animals with intact adrenals.

Exp. 1. Four groups of 10 male rats (Slonaker strain) each, average body weight 270 g were given egg white, 0.5 cc per rat, intraperitoneally. The operations were performed 10 days prior to injections, and the adrenalectomized rats were kept on salt throughout the experiment. The epinephrine-HCl (Parke, Davis natural product), 0.1 mg/kg was injected subcutaneously at the same time as the egg white. The symptoms were graded 4 hours afterward.

Adrenalectomy enhances the toxicity of the egg white and the severity of the symptoms. Epinephrine affords complete protection, in contrast to adrenal cortical extract.[3] Adrenal demedullation alone does not aggravate the symptoms.

Exp. 2. Four groups of 4-5 males each, average body weight 250 g were injected with 1.0 cc egg white per rat. The symptoms were graded 3 hours later. The operations were performed 19 days before the injections, and the adrenalectomized rats were kept on salt until 24 hours before the injections, at which time water was substituted. The epinephrine-HCl, 40 micrograms/kg was injected sub-

TABLE III. Exp. 3.

Group	% incidence of edema	Degree of edema	% dead	Symptoms
1. Untreated controls	100	4.0	0	—
2. Adx (on salt throughout)	100	4.0	80	Cyanotic and prostrated
3. Adx + epinephrine (on salt throughout)	100	4.0	25	< group 2
4. Adx (off salt 1 day)	80	3.5	80	Cyanotic and prostrated
5. Adx + epinephrine (off salt 1 day)	80	2.5	40	< group 3

TABLE IV. Exp. 4.

Group	% incidence of edema	Degree of edema	% dead	Symptoms
1. Untreated controls	100	4.0+	0	—
2. " " + SY-28	0		0	None
3. Adx untreated	100	4.0	80	Cyanotic and prostrated
4. Adx untreated + SY-28	0		0	Prostrated

cutaneously at the same time as the egg white (which is a sub-effective edema-preventative dose.)[1]

Doses of epinephrine which fail to prevent egg white edema even in animals with intact adrenals, completely protect against the fatal effects of egg white intoxication which kills 100% of the untreated adrenalectomized rats. Adrenal demedullation alone does not enhance the edema.

Exp. 3. Five groups of 5 males each, average body weight 180 g, were given egg white 0.5 cc per rat. The symptoms were graded 2 hours later. The adrenalectomies were performed 14 days before the injections, and the animals were maintained on salt both throughout the experiment, and withheld 24 hours prior. The epinephrine-HCl, 0.2 mg/kg was injected subcutaneously at the same time as the egg white injections.

As in Exp. 2 vs 1, intensification of adrenal insufficiency by salt deprivation enhances the susceptibility of adrenalectomized rats, but epinephrine still exerts its protection, in confirmation of the previous two experiments.

Exp. 4. Four groups of 5 males each, average body weight 180 g were given egg white 0.5 cc per rat. The symptoms were graded 2 hours later. The adrenalectomies were performed 14 days before the injections, and the rats were maintained on salt throughout the experiment. "SY-28", a β-haloalkyl amine (dibenamine congener) adrenergic blocking drug, N-(2-bromoethyl)-1-naphthalene-methylamine hydrobromide.‡ was given 10 mg/kg subcutaneously at the same time as egg white.

SY-28 in the dose employed, protects normal and adrenalectomized rats against edema and death, although it was by itself toxic to adrenalectomized rats. "SY-28" is a potent antihistaminic[13,15] in addition to being a very effective adrenergic blocking drug,[13,18] as are several other β-haloalkylamines.[13–15,17,19] Since it is known that antihistaminics inhibit egg white edema in rats with intact adrenals,[1,10,11] it was felt that experiments

‡ Generously supplied by Dr. G. Rieveschl, Jr., Parke, Davis and Co., Detroit, Mich.

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

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

15 Vleeschhouwer, G. R. de, PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 298.

16 Nickerson, M., and Gump, W. S., *J. Pharm. Exp. Therap.*, 1949, **97**, 25.

17 Nickerson, M., and Harris, F. B., *Fed. Proc.*, 1949, **8**, 321.

18 Nickerson, M., *J. Pharm. Exp. Therap.*, 1949, **95**, Part II, 27.

19 Loew, E. R., and Micetich, A., *J. Pharm. Exp. Therap.*, 1949, **95**, 448.

TABLE V. Exp. 5.

Group	% incidence of edema	Degree of edema	% dead	Symptoms
		1 cc egg white		
Adx saline controls	100	2.0	100	Severe cyanosis
" phenergan, 5 mg/kg	60	1.5	20	Cyanosis
" " 10 mg/kg	100	2.0	20	Some cyanosis
" " 25 mg/kg	60	1.0	0	None
		No egg white		
Adx saline controls	—	—	0	"
" phenergan, 5 mg/kg	—	—	0	"
" " 10 mg/kg	—	—	0	"
" " 25 mg/kg	—	—	0	"

should be performed in which an antihistamic as well as a purely adrenergic blocking agent was used in adrenal insufficient animals. As an antihistaminic, Phenergan§ (N-dimethylamino-2-propyl-1-thiodiphenylamine, "32 77-RP") was selected because of its potent antihistaminic action and its anti-capillary permeability effects,[12,20-30] and because in the previous report[1] it was found to be a highly effective inhibitor of egg white edema in the normal rat.

Exp. 5. Eight groups of 5 males each were adrenalectomized 8-10 days prior to the experiment and maintained on salt until 24 hr prior to the experiment, at which time water was substituted. The average body weight at the time of the experiment was 175 g. Phenergan in 2 cc volume was given subcutaneously to the first 4 groups in doses of 5, 10 and 25 mg/kg simultaneously with 1 cc egg white intraperitoneally, and in similar doses to the last 4 groups without egg white, in order to see if the doses of drug given in the absence of egg white were toxic in adrenal insufficient rats. The symptoms were graded 2 hours later.

Phenergan, a potent anthistaminic and anti-capillary "permeability" drug, effectively antagonizes egg white toxicity in adrenal in-

TABLE VI. Exp. 6.

Group (normal rats)	% incidence of edema	Degree of edema
1. Egg white, 1 cc /kg	0	0
2. " " 2 "	0	0
3. " " 3 "	0	0
4. " " 4 "	30	2.0+

‖ In order to rule out the possibility that Phenergan protected *normal* rats against egg white edema, as previously reported,[1] via epinephrine release from the adrenal medulla, experiments were performed on 10 normal and 10 adrenal demedullated rats given 2 cc/kg egg white intraperitoneally, and with and without 2.5 mg/kg Phenergan subcutaneously 1 hour prior to the egg white. There were no differences in the incidence, onset and intensity of the resulting severe edema, in confirmation of our previous work in which it was found that 10 mg/kg was inhibitory.[1] In another experiment on 20 adrenal demedullated rats, however, 25 mg/kg of Phenergan completely inhibited the edema, whereas the untreated animals had marked edema. This experiment therefore rules out the possibility, suggested by the nearly convulsive doses of antihistaminics used by Leger and Masson,[10] and Brown and Werner,[11] that the antihistaminics act by releasing epinephrine from the adrenal glands.

§ Generously supplied by Drs. A. Gibson and R. C. Pogge of Merck and Co., Inc., Rahway, N. J.

20 Halpern, B. N., *J. Allergy*, 1947, **18**, 263.

21 Halpern, B. N., and Cruchard, S., *Compt. rend. Soc. de biol.*, 1947, **141**, 1038.

22 Halpern, B. N., *Acta Allergologica*, 1948, **1**, 3.

23 Halpern, B. N., Hamburger, F., and Cruchard, S., *Acta Allergologica*, 1948, **1**, 97.

24 Halpern, B. N., Guillaumat, L., and Cruchard, S., *Acta Allergologica*, 1948, **1**, 376.

25 Hamburger, J., Halpern, B. N., and Neel, J., *Compt. rend. Soc. de biol.*, 1948, **142**, 183.

26 Halpern, B. N., Guillaumat, L., and Cruchard, S., *Compt. rend. Soc. de biol.*, 1948, **142**, 622.

27 Halpern, B. N., Vermeil, G., and Cruchard, S., *Compt. rend. Soc. de biol.*, 1948, **142**, 1385.

28 Halpern, B. N., and Laubscher, J., *Sem. Hop.*, 1948, **24**, 667.

29 Halpern, B. N., and Hamburger, J., *Canad. Med. Assn. J.*, 1948, **59**, 322.

30 Reuse, J. J., *Compt. rend. Soc. de biol.*, 1948, **142**, 638.

31 Kerwin, J., Ullyot, G. E., Fellows, E. J., and Macko, E., *Fed. Proc.*, 1949, **8**, 308.

TABLE VII. Exp. 7.

Group	Egg white, cc/kg	% incidence of edema	Degree of edema	% dead
1. Normal controls	2	100	3+	0
2. " "	4	100	3	0
3. Normal, + SKF-501	2	83	2	0
4. " "	4	83	2	0
5. Adx controls	2	83	2	33
6. " "	4	83	2	83
7. " + SKF-501	2	100	2	17
8. " + "	4	83	2	33

sufficient rats‖ and does so in doses which are not toxic in adrenalectomized rats not given egg white. As an adrenergic blocking agent without antihistaminic action, "SKF-501,"¶ N-(9-fluorenyl)-N-ethyl-β-chloroethylamine, was chosen since it is an even more powerful yet less toxic adrenergic blocking drug than Dibenamine itself,[31] but has little or no antihistaminic activity,** as might be predicted on the basis that in over 100 compounds tested by Nickerson,[16-18] only α-naphthylmethyl- and β-phenoxyethyl derivatives of the β-haloakylamines were found to be effective antihistaminics.

Exp. 6. Four groups of 3 normal male rats each, average body weight 310 g were given egg white, 1, 2, 3 and 4 cc/kg, respectively, of 1:1 egg white-saline mixture intraperitoneally 3 hours after 2 mg/kg SKF-501 intravenously. The symptoms were graded 2 and 3 hours later.

SKF-501, a purely adrenergic blocking agent, inhibits egg white edema in normal rats.

Exp. 7. Forty-eight male rats were divided into 8 groups of 6 each, with an average body weight of 310 g. The operated groups were adrenalectomized 7 days prior to the experiment and were maintained on salt until 24 hours prior to the experiment, at which time water was substituted. The drug-treated groups were given 2 mg/kg SKF-501 intravenously 1 hour before egg white. The egg white was given in 2 doses, 2 cc/kg and 4 cc/kg intraperitoneally, and the symptoms were graded 3 hours later.

SKF-501, a purely adrenergic blocking drug, decreases the mortality caused by egg white edema in adrenal insufficient rats. The protection in normal controls is somewhat less when given 1 hour before than when given 3 hours before as in Exp. 6.

Discussion. The edema and toxicity induced in normal and adrenal insufficient rats by the intraperitoneal injection of egg white is inhibited by epinephrine and arterenol and probably other pressor substances, but not by Isuprel,†† 1-(3',4'-dihydroxyphenyl) - 2 - isopropylaminoethanol-HCl,[1] (*present paper*). The cephalic edema caused by *p*-phenylenediamine is inhibited by epinephrine, tyramine, phenylethanolamine, drugs which release endogenous epinephrine such as strychnine and nicotine, and by sympathetic stimulation.[32-36] Epinephrine also relieves the edema associated with urticaria and angioneurotic edema. In adrenal insufficiency the increased capillary filtration of fluids induced by traumatic shock, intraperitoneal administration of hypertonic solutions, egg white, and in all probability *p*-phenylenediamine, intravenous hyaluronidase[37] and other conditions, probably all lead to hypotensive shock as a result of an un-

¶ Generously supplied by Dr. Glen E. Ullyot of Smith, Kline and French Laboratories, Philadelphia, Pa.

** As tested by the prevention of histamine-induced spasm of the isolated guinea pig intestine.[16]

†† Not investigated in adrenalectomized rats.

32 Hanzlik, P. J., *J. Ind. Hyg.*, 1923, **4**, 386.

33 Tainter, M. L., and Hanzlik, P. J., *J. Pharm. Exp. Therap.*, 1924, **24**, 179.

34 Gibbs, O. S., *J. Pharm. Exp. Therap.*, 1923, **20**, 221; 1931, **42**, 65.

35 Tainter, M. L., *J. Pharm. Exp. Therap.*, 1926, **27**, 201; 1928, **33**, 129.

36 Cohen, M. B., Wasserman, P., and Rudolph, J. A., *J. Pharm. Exp. Therap.*, 1933, **48**, 235.

37 Elster, S. K., Freeman, M. E., and Dorfman, A., *Am. J. Physiol.*, 1949, **156**, 429.

compensated decreased blood volume. Epinephrine may antagonize all of those effects by its arteriolar vasoconstrictive action; and in addition protects adrenalectomized animals against histamine and anaphylactic shock. Antihistaminics inhibit egg white edema, hyaluronidase edema,[38] urticarias, angioneurotic edema and histamine and anaphylactic shock probably both by antagonizing the action of released histamine and by decreasing capillary filtration by arteriolar vasoconstriction. No explanation can be given for the protective effect of adrenergic blockade in egg white edema. At first thought, it might have been anticipated that it would aggravate the condition by blocking the protective action of endogenous epinephrine and sympathin-N. This is not the case, however, and the fact the adrenal demedullation also is not detrimental is confirmatory of this lack of effect. It is possible that SFK-501 and the adrenolytic component of SY-28 protect in the same way that Dibenamine does in hemorrhagic and traumatic shock, as demonstrated by Remington,[39,40] although the mode of action in both cases is unknown. The effect suggests, however, the participation of an adrenergic component in egg white edema, as seems to be the case in the pulmonary edemas induced by ammonium chloride[41,42] and increased intracranial pressure or brain concussion.[43]

Summary. Epinephrine protects adrenal insufficient rats from the edema and mortality caused by the intraperitoneal administration of egg white. Mortality is prevented by doses (0.04 mg/kg) which are not able to prevent edema in rats with intact adrenals.

The antihistaminic Phenergan, N-dimethylamino-2-propyl-l-thiodiphenylamine ("3277 RP"), in doses as low as 5 mg/kg prevent death from egg white edema in adrenalectomized rats.

"SY-28", N-(2-bromoethyl)-1-naphthalenemethylamine hydrobromide, a Dibenamine congener possessing both antihistaminic and adrenergic blocking activity, also prevents the edema and death, but is in itself toxic to adrenalectomized rats.

"SKF-501", N-(9-fluorenyl)-N-ethyl-β-chloroethylamine, a Dibenamine cogener with potent adrenergic blocking powers but with a low toxicity and without antihistamine action, also inhibits the edema and decreases the mortality.

The possible modes of action are discussed.

[38] Elster, S. K., Freeman, M. E., and Lowry, E. L., *J. Pharm. Exp. Therap.*, 1949, **96**, 332.

[39] Remington, J. W., Wheeler, N. C., Boyd, G. H., Jr., and Caddell, H. M., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 146.

[40] Remington, J. W., *Fed. Proc.*, 1949, **8**, 131.

[41] Koenig, H., and Koenig, R., *Am. J. Physiol.*, 1949, **158**, 1.

[42] MacKay, E. M., Jordan, M., and MacKay, L. L., PROC. SOC. EXP. BIOL. AND MED., 1949, **72**, 421.

[43] MacKay, E. M., in press.

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A Microbiological Assay for Vitamin B_{12} Using *Lactobacillus leichmannii*.* (17485)

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Lactobacillus leichmannii (ATCC 4797) has been reported by several laboratories[1-3] to be a useful test organism for the microbiological measurement of vitamin B_{12}. Skeggs, *et al.*[1] found ascorbic acid and air to stimulate a growth response in this organism in the absence of vitamin B_{12} on a basal medium containing casein hydrolysates, but were able to minimize these effects by autoclaving the test assay for 15 minutes at 120°C. Other workers[3] also found certain reducing agents to promote a growth response in both *L. leichmannii* (ATCC 4797) and *L. leichmannii* (ATCC 7830) in the absence