of hemoglobin as well as sickling hemoglobin is not readily apparent. Neel(8) has suggested that persistence of genes in the course of evolutionary development should result from some positive contribution to fitness. Perutz *et al*(16), in discussing the biologic advantage of heterogeneity of hemoglobin in horses, has pointed out that the solubility of oxyhemoglobin in the horse is low and that by the phase rule, a saturated solution of 2 fractions should contain more protein than a saturated solution of either. The coexistence of various hemoglobin components in horses might therefore permit a higher concentration of hemoglobin without crystallization within the cell. The mean corpuscular hemoglobin concentration in all the deer examined was 36%. Since the blood values in deer with single hemoglobin components were essentially the same as those with several hemoglobin components, it seems unlikely that heterogeneity is necessary to prevent crystallization of hemoglobin within deer erythrocytes.

Summary. A marked degree of heterogeneity is demonstrable in deer hemoglobin. This heterogeneity appears to be on a genetic basis. A single hemoglobin component responsible for sickling of deer hemoglobin can be identified.

Addendum. Since this paper was prepared, similar electrophoretic results were reported by Kitchen, H., Putnam, F. W. and Taylor, W. J., in *Science,* 1964, v144, 1237.

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On the Anti-Inflammatory Activity of Aminophylline. (29557)

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Aminophylline, theophylline ethylene diamine, is one of the most effective bronchodilating agents available and it plays a very pertinent role in the management of the asthmatic patient(1). On theoretical grounds it would appear desirable that a bronchodilator possess, in addition to its smooth muscle-relaxing-property, a degree of anti-inflammatory or antiedemic activity to enable it to reduce airway obstruction due to engorged or swollen epithelial linings. In testing aminophylline in several laboratory models of inflammation, or local edema, it has become apparent that this xanthine derivative does indeed possess a significant degree of inhibitory activity in several models of increased capillary permeability.

Methods. Rat. Wistar rats of either sex, weighing 140-160 g each, were employed in these models of pedal edema which was measured plethysmographically by mercury displacement as described previously(2). Rats

TABLE I. Effect of Aminophylline on Certain Models of Pedal Edema in Rats.

Dose* mg/kg	.5% Carrageenin — Mean %† increase in edema ± S.E.	% Inhibition	6% Dextran — Mean %‡ increase in edema ± S.E.	% Inhibition	4% Formalin — Mean %‡ increase in edema ± S.E.	% Inhibition	.004%§ Serotonin — Mean %‡ increase in edema ± S.E.	% Inhibition
0 (Control)	43.6 ± 2.7 (16)	—	74.4 ± 2.8 (29)	—	38.7 ± 2.1 (20)	—	38.1 ± 2.0 (20)	—
50	36.7 ± 4	15.8	63.7 ± 2.6 (20)	14.4	38.2 ± 2.7	1.3	35.3 ± 1.8	7.4
100	35.4 ± 2.9	18.8	65.3 ± 5.9	12.2	36.6 ± 2.4	5.5	38.2 ± 2.1	0
200	25.2 ± 3.8	42.2	50.0 ± 4.2 (20)	32.8	32.2 ± 2.8	16.8	35.9 ± 2.7	5.8
400	24.5 ± 2.6	43.8	38.4 ± 6.9	48.4	18.5 ± 3.0	52.2	30.0 ± 1.4	21.3

* Given orally in 0.9% NaCl 30 min prior to edemogen; 10 rats per group, except where indicated (#).
† Mean of group; mean % increase in volume of edemogen-treated foot *vs* saline-injected foot, determined plethysmographically 3 hr following edemogen-injection.
‡ As † except edema determined 1 hr following edemogen.
§ Expressed as serotonin base (serotonin creatinine SO_4 = .0092%).

were divided into groups of 10 each and treated with varying dose levels of aminophylline orally 30 minutes (60 minutes in the carrageenin model) prior to induction of edema by the subplantar injection of 0.1 ml of the particular edemogen solution or suspension into the right hind foot. The contralateral foot received 0.1 ml of saline. The carrageenin edema method, originally described by Winter *et al*(3), was modified somewhat. A 0.5 % suspension of carrageenin* in 0.9% NaCl was employed. Other edemogens were 6% dextran (Pharmacia), 4% formalin, and 0.004% serotonin base (or 0.0092% as serotonin creatinine sulfate; Nutritional Biochemicals Corp.).

The extent of edema was determined one hour later (3 hours later with carrageenin) and is reported as the mean % increase in edema of the edemogen-injected foot over the contralateral saline-injected foot. The per cent inhibition of edema in the treated groups is expressed relatively to nontreated control groups.

Guinea pig. Erythema was induced on guinea pig skin by direct exposure to ultraviolet light according to a modification(4) of the method described earlier by Winder *et al*(5). Hair was removed from pigs of either sex weighing 300-400 g the afternoon prior to use. On the day of the test they were treated orally with aminophylline 30 minutes prior to exposure of 60 seconds. Two hours after exposure the extent of erythema development on 3 sites was assessed by a 0-5 scoring basis, so that each pig could score a maximum of 15. The score is reported as the mean score per pig for a group of 5 pigs, and the degree of inhibition in the treated-groups is compared against a similarly-exposed, nontreated control group.

Results. It is apparent from the data in Table I that aminophylline did possess a degree of antiedemic activity against certain models of increased capillary permeability in the rat paw. The end-result of these variously-induced edemas is similar in gross appearance, but the mechanism and chemistry of the development of each model, and in turn the response to an inhibitor, differs. Both dextran- and formalin-induced edemas were of approximately equal sensitivity to inhibition by aminophylline. From log dose-response lines the estimated dose required to inhibit either model by 50% was approximately 400 mg/kg. But the maximum inhibition of carrageenin apparently attainable with this drug appeared to be 42-44% at both the 200 and 400 mg/kg dose levels. Thus, carrageenin-induced edema was somewhat less susceptible than either dextran or formalin to inhibition by aminophylline. Since a dose of 400 mg/kg inhibited by only 21.3%, it is further evident from Table I that aminophylline was least effective against serotonin-induced edema.

On the basis of the data in Table II it is apparent that aminophylline also possessed some antierythemic activity in the guinea pig. However, the drug was somewhat more

* "Kraystay"; obtained from National Dairy Products Corp., Kraft Foods Division, Chicago, Ill.

TABLE II. Effect of Aminophylline on Ultraviolet Erythema in Guinea Pigs.

Dose* mg/kg	Mean erythemic response ± S.E.†	% Inhibition
0	10.6 ± .7	—
50	10.0 ± 1.0	5.7
100	7.8 ± .7	26.4
200	6.2 ± 1.0	41.5
400	5/5 died	

* Given orally in .9% NaCl 30 min prior to exposure to UV light; 5 pigs per group.

† Mean score per pig in groups of 5 pigs; 3 erythemic spots per pig; scored on 0-5 basis, maximum score per pig = 15; 2 hr after exposure.

toxic acutely to this species than to rats. From the log-dose response line in this model the dose of aminophylline estimated to be required to inhibit erythema development by 50% was about 270 mg/kg. This dose is only some 65% of that required for an equivalent degree of antiedemic activity in the rat (Table I).

Discussion. Under the conditions of these experiments aminophylline evidenced a somewhat unexpected degree of anti-inflammatory activity. From the carrageenin-induced edema which is sensitive to the principal classes of clinically-useful anti-inflammatory drugs (aspirin, phenylbutazone, and hydrocortisone(3)) and from the ultraviolet light-induced erythema which is inhibited by both non-steroidal agents(4,5) it might seem that aminophylline does possess a true anti-inflammatory component, mild though it might be, to its spectrum of biologic activity.

On the basis of the data for aspirin and phenylbutazone reported by Winter *et al*(3) aminophylline is only about one-half as potent as the former and one-fourth as active as the latter in the carrageenin edema model. Data obtained with those same drugs in that model in this laboratory tend to agree. In addition, aminophylline is about one-third as potent as aspirin against ultraviolet erythema in the guinea pig(4,5). Also the xanthine derivative is only 5-6% as active as the very effective phenylbutazone in this model.

Furthermore, the inhibition of formalin-induced edema suggests that an antiedemic mechanism, aside from an antihistamine or antiserotonin action, may exist for aminophylline. This possibility is further corroborated by the limited antagonism of serotonin-induced edema in spite of the effectiveness against dextran(6,7). Since dextran edema is mediated by serotonin release(6,7) and since aminophylline inhibited this edema but not that produced by serotonin itself, the xanthine derivative may act in the dextran model at some point prior to release of the mediator. For example, aminophylline may prevent serotonin release. Such a possibility is consistent with the inhibition by this drug of the release of slow-reacting substance (SRS-A) and histamine in anaphylactic shock in guinea pigs reported by Firth and Smith(8). Thus, considering its inhibition of 3 laboratory models of inflammation (carrageenin- and formalin-induced rat paw edema and guinea pig erythema) which are known not to depend on release of a biogenic amine, it appears that aminophylline may possess some specific antiedemic or anti-inflammatory property.

One might wonder to what extent the smooth muscle relaxant and/or vasodilator property in aminophylline contributes to the antiedemic activity noted in this study. At least it can be said that the oral doses employed in this report are of the same order as the subcutaneous doses reported by Dungan and Lish(9) to be effective in increasing the "pre-dyspneic interval" in guinea pigs exposed to a histamine aerosol. Furthermore, one might also speculate as to what extent the antiedemic activity apparent in this present investigation might contribute to the therapeutic benefit obtained with aminophylline in bronchoconstriction and pulmonary disease.

Summary. When given orally aminophylline exhibited significant inhibition of at least 3 of 4 models of increased capillary permeability in the rat paw. The sensitive models were those induced by carrageenin, dextran, and formalin. Serotonin was affected at only the highest dose level (400 mg/kg). A fourth model, ultraviolet light-induced erythema in guinea pigs, was also inhibited.

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Histamine in a Transplantable Mouse Mastocytoma.* (29558)

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It has been well established by Riley and West(1) that mast cells contain histamine. High histamine values have been demonstrated in mastocytoma of various species(2, 3,4,5) and in urticaria pigmentosa(3,5,6,7, 8), the skin manifestation of human mastocytosis. Mast cell tumors, however, are rare. Three types of transplantable mastocytoma in mice have been reported(9,10,11).

The present paper reports on analyses for the histamine content in the tumor and various organs of a mouse with a transplantable mastocytoma of the third type.

Materials and methods. The primary tumor(11) arose in a (CBAxDBA/2)F_1 mouse that had been inoculated subcutaneously with leukemic tissue from a transplantable plasma cell leukemia. There was no visible growth of the inoculated material. The primary and the transplanted tumors consisted of mast cells. The ultrastructure has previously been studied by electron microscopy (12). A female mouse from the first passage in which tumor tissue had been inoculated intraperitoneally showed local growth consisting of soft, whitish, flabby tissue located to the mesentery. Eighteen months had elapsed from time of inoculation till the animal was killed.

Histamine was determined by the spectrofluorometric method(13) in dried and defatted tumor tissue and skin. Histamine was also determined in fresh skin, liver, spleen and ovary. The specimens were obtained immediately after the animal was killed and frozen at —20°C until they were analyzed. The subcutaneous fat was mechanically removed from the skin proper by knife and scissors. Defatting was carried out by shaking the minced samples twice with 15 ml of ether for one hour. The defatted tumor and skin tissue was then lyophilized for 48 hours at room temperature under a pressure of 2 mm Hg. The defatted and dried and the fresh samples were homogenized in 5 ml of 0.4 N perchloric acid using a motor-driven glass homogenizer. The homogenate was allowed to stand for 10 minutes and then centrifuged. A 4 ml aliquot of the supernatant was analyzed for histamine according to the procedure previously described(14).

Results. Table I shows the histamine content of the tumor and of the various organs of the tumor mouse. All values represent histamine base.

Comments. The histamine content of the mastocytoma induced by inoculation was high in comparison with some of the scarce

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