Summary. When serum is subjected to electrophoresis at pH 4.5, two components are seen which have isoelectric points more acid than those of albumin. One of these has been identified with a mucoprotein which has been shown to increase in amount in cancer, pneumonia, and certain other diseases. The second component, with a less acid isoelectric point, may also be a mucoprotein, but this point has not been definitely established.

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Effect of Adrenergic Blocking Agents on the Cutaneous Action of Epinephrine. (17349)

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Adrenergic blocking drugs are of clinical interest in the treatment of conditions characterized by the element of vasospasm, such as early Buerger's disease, Raynaud's disease and causalgic states. It was felt that by the direct diffusion or by ion transfer through the skin, it would be possible to avoid the generalized toxic manifestations encountered by way of the parenteral route.[1] The adrenergic blocking agents selected for this study were N-(2-chloroethyl) dibenzylamine hydrochloride (Dibenamine) introduced by Nickerson and Goodman[2] and N-(2-bromoethyl)-N-l-naphthalenemethylamine hydrobromide (SY-28) introduced by Loew and Micetich.[3] Prior to application in the human, data were obtained in a group of albino rabbits.

Methods. Albino rabbits weighing 2.0 to 4.5 kg were used. The abdomen and thorax of the animals were depilated with a special sulfide-detergent mixture[4] 24 hours prior to use. Solutions of Dibenamine and SY-28 in propylene glycol were freshly prepared prior to each experiment. Four-tenths ml of these solutions or of solvent controls were applied to rectangular strips of asbestos paper 1.25 by 12.5 cm in size. This amount of solution was sufficient to saturate the pads. The strips were then applied to the abdomen in 4 parallel sites in the following order: solvent without current, solution containing the drug without current, solution containing the drug plus current, solvent plus current. Two to four rabbits were used at each of the various concentrations of blocking agents studied. Since the active portions of both Dibenamine and SY-28 are cationic, the anode of a suitable direct current source* was used to transfer the agent into the skin. A current density of 0.33 ma/cm^2 or a total current of 5.2 ma was employed. This dosage of current was arbitrarily selected as being just below the pain threshold for human skin and was well tolerated by the rabbits. The solutions were placed in contact with the skin for exactly 5 minutes with or without current. Immediately following the 5 minute application, the asbestos pads were removed and the blocking agent wiped off with propylene glycol.

To measure the adrenergic blocking activity of the treated sites, 0.1 ml of 1:1,000, 1:10,000 and 1:40,000 epinephrine solutions were injected intracutaneously 30 minutes following application of the blocking agents. An equal amount of physiological saline served as a control. The injection sites were randomized. Vasoconstriction reached a max-

[1] Hecht, H. H., and Anderson, R. B., *Am. J. Med.*, 1947, **3**, 3.

[2] Nickerson, M., and Goodman, L. S., *Proc. Am. Fed. Clin. Research*, 1945, **2**, 109.

[3] Loew, E. R., and Micetich, A., *Fed. Proc.*, 1947, **6**, 304.

[4] Pitesky, I., and Last, J. H., *Science*, 1948, **108**, 657.

* A McIntosh Wall Plate was used. This machine is manufactured by the McIntosh Electrical Corp., Chicago, Ill.

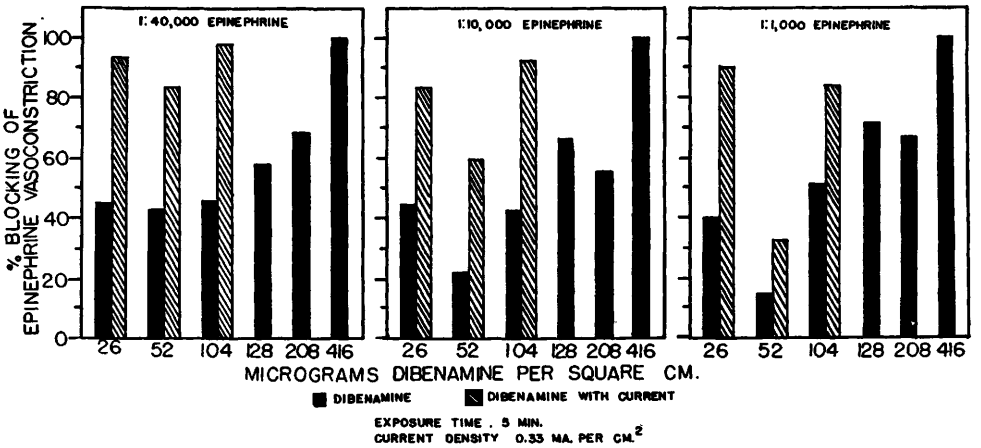

FIG. 1.

Effects of ion transfer on the cutaneous adrenergic blocking activity of Dibenamine.

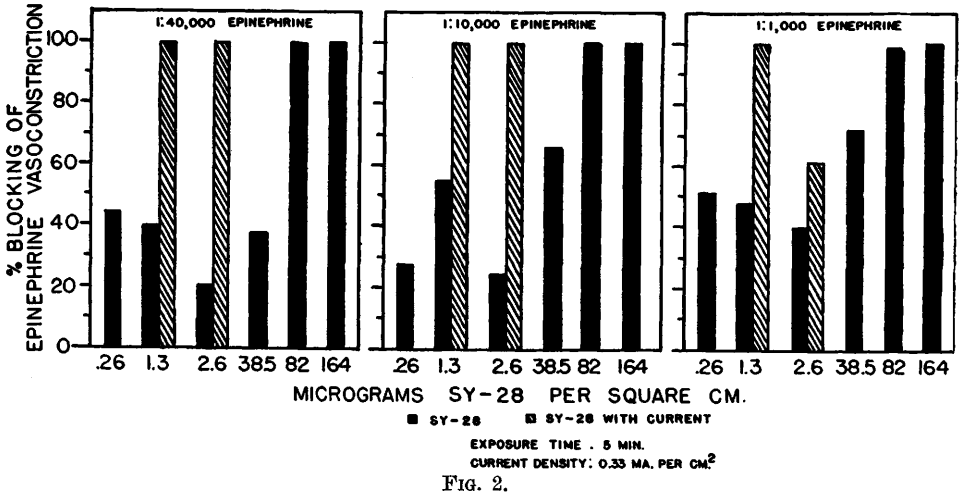

FIG. 2.

Effects of ion transfer on the cutaneous adrenergic blocking activity of SY-28.

imum in 30 minutes and manifested itself by a circular area of blanching.

The diameters of the blanched skin were measured and the areas computed. These areas were then weighted for intensity of vasoconstriction. This was accomplished by arbitrarily recognizing four degrees of response and assigning an intensity number for each degree. Full, faint, very faint, and no vasoconstriction, (*i.e.* complete blocking), were assigned the numbers 3, 2, 1 and 0 respectively. The area multiplied by the intensity factor yielded a weighted area of vasoconstriction. The percentage of blocking was then easily obtained mathematically by comparing the treated sites with the control sites. The results were analyzed statistically by the method of paired comparisons. After calculating the statistic "t", the probability value "P" was obtained from Fisher's "t" table.

Results. The data obtained for Dibenamine and SY128 are illustrated in Fig. 1 and 2. Since the absolute amount of drug present in the skin following exposure was unknown, the values plotted along the abscissae were the amounts actually applied to the skin.

Dibenamine produced complete blocking of epinephrine at a dose of 416 μg per sq cm whereas SY-28 produced the same effect with 82 μg per sq cm. Under these conditions SY-28 was approximately 5 times as potent as Dibenamine in blocking epinephrine. The ap-

plication of current enhanced the penetration of both drugs in a statistically significant manner. Analysis of the epinephrine responses in 4 rabbits following the use of Dibenamine in a concentration of 104 $\mu g/cm^2$ with and without ion transfer yielded the following "P" values at various epinephrine concentrations:

a. 1:1,000 - P = 0.01
b. 1:10,000 - P = 0.06
c. 1:40,000 - P = 0.04

Correspondingly, with SY-28 in 4 rabbits with and without ion transfer at a dosage of 1.3 $\mu g/cm^2$ the following P values were calculated for the concentrations of epinephrine used:

a. 1:1,000 - P = 0.04
b. 1:10,000 - P = 0.07
c. 1:40,000 - P = <0.01

Dibenamine and SY-28 were found to be fixed locally, since the skin surrounding the treated site responded to injected epinephrine in a normal manner. During the course of the study, it was noticed that an area of erythema developed in the sites injected with epinephrine prior to the appearance of vasoconstriction. This response was even more marked and persistent when vasoconstriction was completely blocked. The process of ion transfer produced minimal erythema which made it difficult to evaluate the subsequent erythematous response to epinephrine. For this reason two groups of 8 rabbits each were given Dibenamine or SY-28 by the intravenous route. Epinephrine was injected intracutaneously as in the previous experiments 30 minutes after the injection of the blocking drug. Erythema developed rapidly and either disappeared over a 15 minute period or was followed by vasoconstriction in the situations where incomplete blocking occurred. This "epinephrine reversal" type of phenomenon suggests the presence of vasodilator fibers in the skin of the rabbit.

Fig. 3 and 4 illustrate a dose response relationship when the blocking agents were given intravenously. One hundred percent blocking of epinephrine vasoconstriction occurred when 32 mg of Dibenamine per kilo were given. The same effect was achieved with SY-28 when 4 mg per kilo were injected. Thus

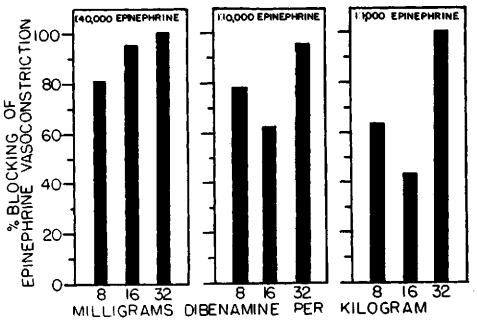

FIG. 3.
Cutaneous adrenergic blocking activity of intravenous Dibenamine.

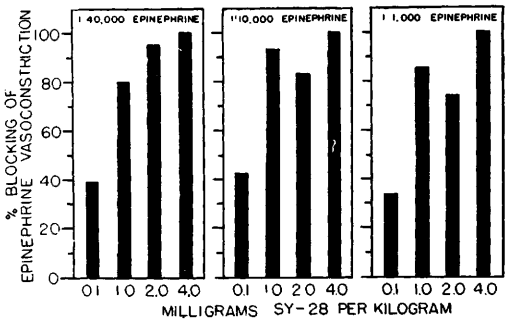

FIG. 4.
Cutaneous adrenergic blocking activity of intravenous SY-28.

SY-28 was approximately 8 times as potent as Dibenamine when given by the intravenous route.

Summary. 1) Both Dibenamine and SY-28 were absorbed by the intact rabbit skin and were capable of blocking the vasoconstriction produced by intracutaneously administered epinephrine. By this route SY-28 was approximately 5 times as potent as Dibenamine.

2) When administered by ion transfer, the penetration of both drugs into the skin was significantly facilitated.

3) Both blocking compounds were fixed locally in the skin. Contiguous untreated areas were still capable of responding to epinephrine.

4) By the intravenous route, SY-28 was approximately 8 times as potent as Dibenamine.

5) The "epinephrine reversal" phenomenon has been noted for the first time in the skin of the rabbit and suggests the presence of vasodilator fibers.

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