must be assumed that the tonic neurogenic constriction of afferent arterioles is greater than that of efferent arterioles. This interpretation is a variance with the conclusion of Chasis, *et al.*[12] that the efferent arteriolar bed is the major site of vasomotor regulation in the human kidney, and the conclusion of Hiatt[13] that the renal circulation of the dogs has little or no vasomotor tone.

It is clear that our present knowledge of the complex mechanism controlling renal blood flow is inadequate to provide a definitive explanation of the mechanism by which Dibenamine increases the renal filtration fraction. It is possible that in our experiments the lowered blood pressure and renal blood flow after Dibenamine induce a sufficient production of renin to alter the picture of pure vasomotor blockade. Additional experimental work is required to elucidate the mechanism of the Dibenamine induced increase in filtration fraction. Clarification of this problem may also provide a better understanding of renal vasomotor regulation.

Summary. 1. Six weekly intravenous injections of Dibenamine (20 mg/kg) failed to produce any detectable renal damage as measured by creatinine and PAH clearances in 5 trained, unanesthetized female dogs, even when the entire dose was injected within one minute so as to produce maximum toxic side-effects including prostration for 24 to 48 hours.

2. During maximal sympathetic blockade by Dibenamine, renal plasma flow was reduced 10 to 34% (average 21%), probably on the basis of reduced systemic arterial pressure. However, the filtration fraction was sufficiently elevated to provide unaltered glomerular filtration. These alterations never persisted beyond the period of active adrenergic blockade.

3. We have been completely unable to substantiate the observations of Ogden[7] on the renal toxicity of Dibenamine or to find any renal basis for his warning regarding its use. Even large doses of Dibenamine administered very rapidly appear to have no significant reno-toxic action.

12 Chasis, H., Ranges, H. A., Goldring, W., and Smith, H. W., *J. Clin. Invest.*, 1938, **17**, 683.

13 Hiatt, E. P., *Am. J. Physiol.*, 1942, **136**, 38.

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Enhancement of Penetration of Penicillin into Inflamed and Normal Mucous Membrane by Hyaluronidase.

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Duran-Reynals[1] first described the presence in testicular extract of a factor which is capable of facilitating the spread of vaccine virus in the skin of rabbits. Since then, this factor has been shown to be identical with hyaluronidase.[2] The enzyme has been found capable of spreading such diverse substances as vaccine virus, dyes, india ink, rabies virus, hemoglobin, diphtheria toxin, glucose and methemoglobin. It appeared to the authors that it would be of interest to determine whether penetration of penicillin into a mucous membrane might also be enhanced by hyaluronidase particularly since therapeutic failure with penicillin is sometimes ascribed to the inability of the antibiotic to reach deep-seated foci of infection. Our studies were carried out as follows:

1 Duran-Reynals, F., *Compt. Rend. Soc. Biol.*, 1948, **99**, 6.

2 Chain, E., and Duthie, E. S., *Brit. J. Exp. Path.*, 1940, **21**, 324.

TABLE I.
Blood Penicillin Levels in Patients with Chronic Suppurative Sinusitis Following the Instillation of 200,000 Units of Crystalline Penicillin G into the Antrum. With and Without Hyaluronidase. (Units/ml serum.)

Patient	Hyaluronidase	Time after instillation 30M	60M	120M
1.	0	1.33	.8	
2.	0	.8	.57	
3.	0	1.33	.8	
4.	0	.8	.44	
5.	0	.133	.08	
6.	0	1.0	.8	
7.	0	.57	.4	
8.	0	.44	.133	
9.	0	1.33	1.0	
10.	0	1.0	.66	
11.	0	.8	.44	
12.	0	.2	.2	
13.	0	1.0	.8	
14.	0	.8	.5	
15.	0	.1	.066	
	H-1	.66	.4	
16.	0	.5	.44	
	H-1	1.33	1.0	
17.	0	.57	.44	
	H-1	1.0	.66	
18.	0	.5	.44	
	H-1	1.0	.88	
19.	0	< .05	< .05	
	H-11	.4	.133	
20.	0	.1	.2	.2
	H-11	.5	.5	.44
21.	0	< .05	< .05	
	H-11	.1	.066	
22.	0	—	.2	< .05
	H-11	.2	.2	.2
23.	0	.8	.57	
	H-11	1.0	.8	
24.	0	.2	.1	
	H-11	.5	.4	
25.	0	1.0	.8	
	H-11	.8	.57	
26.	0	.2	.133	
	H-11	.4	.2	

Experimental. Two groups of patients were selected for this investigation. One group consisted of 26 patients with varying degrees of chronic suppurative disease of the maxillary sinus. The other consisted of 5 subjects clinically free of sinus disease in whom antral lavage yielded a clear return.

After preliminary cocainization followed by suction and cleansing, an antral cannula was inserted into the maxillary sinus through the middle meatus. The sinus was then irrigated with physiological saline solution and the excess fluid was drained off as far as possible by tilting the head so as to make the natural

TABLE II.
Blood Penicillin Levels in Normal Subjects Following the Instillation of 200,000 Units of Crystalline Penicillin G into the Antrum. With and Without Hyaluronidase. (Units/ml serum.)

Patient	Hyaluronidase	Time after instillation		
		30M	60M	120M
A	0	.4	.08	
B	0	1.3	1.3	
C	0	.44	.2	.05
	H-11	.66	.5	.4
D	0	.5	.4	.057
	H-11	1.0	.8	.57
E	0	.5	.44	.1
	H-11	.8	.66	.3

ostia dependent. Air under 15 lb pressure was blown into the cavity in order to further empty the sinus. 200,000 units of crystalline penicillin G, dissolved in 2-3 cc of saline solution, were instilled into the sinus. Large doses were employed in order to achieve adequate blood levels so that comparisons with and without hyaluronidase would be definitive. With the crystalline preparation unlike the older amorphous penicillin local irritation was absent or minimal. The head was now tilted towards the irrigated side and kept in this position throughout the period of observation in order to prevent escape of the antibiotic. After the cannula was withdrawn a gelfoam pack was inserted into the middle meatus as an additional safeguard against leakage. In the first 3 patients tested the instilled penicillin was colored with gentian violet and upon removal of the white gelfoam pack, the absence of any stain upon it was an indication that leakage had not occurred.

Blood samples at 30, 60 and in some instances 120 minutes after the antral instillation were drawn from the antecubital vein for penicillin assay. The blood penicillin level was considered a good index of penetration since the only way in which the antibiotic could reach the blood stream was by traversing the mucous membrane. The method of assay was a tube dilution method using *Staphylococcus aureus* H as the test organism and fresh meat extract broth as the medium. The minimal concentration of standard penicillin* required to inhibit an inoculum of 5×10^2 *Staphylococcus aureus* H cells was 0.02 units per ml. All titrations of serum levels were compared with this standard.

The effect of hyaluronidase on penicillin penetration was investigated in a number of patients in each group by administering after a few days the same dose of penicillin with the addition of hyaluronidase. Blood samples were again assayed for penicillin and the values found with hyaluronidase and without its use were compared. Two batches of hyaluronidase† were utilized. The first, designated H-1, was employed in a dose of 20 turbidity reducing units. The second, H-11, was given in doses of 42 units since preliminary trials revealed this batch to be not as potent as H-1.

Results. The instillation of large doses of crystalline penicillin into the diseased and normal antrum was well tolerated by all the patients under study. There were no subjective complaints and no evidence of local irritative reaction following treatment. One patient proved to be allergic to penicillin and 24 hours after the instillation exhibited a diffuse skin eruption with edema of both lids. He readily responded to treatment with an antihistamine drug. On further questioning the patient recalled having experienced a similar reaction when penicillin had been administered elsewhere by the parenteral route. Thus it was found that large doses of crystalline penicillin might be safely instilled into the antral cavity

* Obtained from the U. S. Department of Agriculture.

† Kindly supplied by The Schering Corporation.

provided the usual precautions in respect to allergic reactions to the drug were observed.

As may be noted from Table I, a significant blood penicillin level was present in 24 out of 26 cases with chronic suppurative sinusitis following the intra-antral instillation of 200,000 units of the crystalline product. In every instance except one (Patient No. 25) the blood level was uniformly higher after the addition of hyaluronidase to the penicillin solution. In general, the blood levels were found to be about 2 to 3 times greater than those found without its use. Furthermore, it is of interest that patients No. 19 and No. 21 in whom no evidence of absorption could be demonstrated, showed significant levels after the addition of hyaluronidase. These findings suggest that hyaluronidase increases diffusion and penetration of penicillin through the diseased antral mucosa.

Similar findings to those noted above were obtained in subjects with non-diseased mucous membranes (Table II). A significant level was found in the blood of all 5 subjects after the antral instillation of penicillin and in each patient tested, the addition of hyaluronidase resulted in higher levels.

Although we were not primarily concerned in this study with the evaluation of the clinical effectiveness of large doses of crystalline penicillin supplemented by hyaluronidase in the treatment of chronic suppurative disease of the maxillary sinus, it is of interest that marked clinical improvement occurred in some of the patients.

Summary. The instillation of 200,000 units of crystalline penicillin G into the diseased and normal paranasal antrum is well tolerated and except for the development of an allergic reaction in one patient, was without any adverse effect. In 24 out of 26 patients with chronic suppurative disease of the sinuses and in all 5 normal subjects, a significant penicillin level in the blood was found after the intra-antral instillation.

In both groups, the addition of hyaluronidase to the instilled penicillin resulted in even higher blood levels than those found without its use, with one exception. In two patients in whom no blood level could be demonstrated, the addition of hyaluronidase resulted in a significant concentration of penicillin in the blood. It is postulated that the increased blood penicillin level following hyaluronidase is due to greater diffusion and penetration of the penicillin as a result of the spreading action of hyaluronidase.

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Tuberculin Reaction. III. Transfer of Systemic Tuberculin Sensitivity with Cells of Tuberculous Guinea Pigs.*

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In spite of many years of research by numerous investigators the passive transfer of tuberculin sensitivity was not accomplished with any degree of certainty or regularity until the important work of Chase[1] whose technic has provided a new means of exploring many aspects of this highly important type of sensitivity.†

In previous publications,[2,3] we have pre-

* This investigation was supported by a grant from the United States Public Health Service.

1 Chase, M. W., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 134.

† The term sensitivity is used throughout the present article in preference to the more commonly used term hypersensitivity.

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