Microscopic examination. All animals of Groups 3 and 4 were examined, but in the larger groups, 25 sets of sections were selected at random from each group for examination. Study of Ziehl-Neelsen and hematoxylin-eosin stained sections disclosed no difference between the controls (Group 1) and thonzylamine treated animals (Group 2) with respect to type of lesions or to numbers of bacilli present. All animals in these two groups showed predominantly fibrotic lesions with moderate caseation which contained small numbers of acid fast bacilli. The streptomycin treated guinea pigs (Group 4), and those receiving both drugs (Group 3) showed more fibrosis, fewer caseating lesions, and bacilli were difficult to find. The lesions in the latter two groups were similar to the lesions described by Karlson and Feldman(3) in tuberculous guinea pigs given the same dose of streptomycin.

Discussion. Thonzylamine, an effective antihistaminic agent which is capable of suppressing allergic reactions, was used in this and in our preceding study in an effort to determine whether or not it would aid in the chemotherapy of infectious diseases. It is evident from the above experiment that thonzylamine causes a slight but statistically significant increase in the survival time of experimentally infected tuberculous guinea pigs, and this appears to be reflected in a lessened spread of the disease. With curative doses of streptomycin (unreported experiments) the disease responded promptly and no effect attributable to thonzylamine could be discerned. With sub-curative doses of the antibiotic, our findings were in agreement with those reported by Karlson and Feldman(3) but, here again, thonzylamine did not influence nor aid streptomycin therapy. It must be concluded that thonzylamine is not a practical aid in the therapy of experimental tuberculosis in guinea pigs, but the slight beneficial result still leaves open the possibility that antihistaminic drugs may be useful in combatting the allergic component of infectious diseases.

Conclusions. Thonzylamine caused a slight reduction in the extent of tuberculosis in experimentally infected guinea pigs and caused a slight but significant increase in the length of survival. It did not supplement the effects of streptomycin.

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Evidence that Hyaluronidase is Not the Factor in Testicular Extract Causing Increased Vascular Permeability.* (18877)

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A factor capable of increasing vascular permeability was demonstrated to be present in bull testis extracts by Duran-Reynals(1). This finding has been amply confirmed(2-5). Duran-Reynals and others have assumed that hyaluronidase is responsible for both spreading and increase in vascular permeability.

We have investigated hyaluronidase preparations, more highly purified than those previously available, for vascular permeability-increasing activity. Two approaches were used: (1) a study of the local extravasation of an intravascular colloidal dye following

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TABLE I. Influence of Purification of Testicular Hyaluronidase upon its Capacity to Increase Vascular Permeability in Rats.

Exp No.	Hyaluronidase: Prep-aration	Hyaluronidase: Purity,† μ/mg N	Evidence of increased vascular permeability: Evans Blue conc, μg/ml blood corrected for body wt		Hemoglobin conc, g/100 ml		Visible edema and blueing
			Saline	Enzyme	Saline	Enzyme	
1	A	1,000	134 ± 2.7*	82 ± 5.3*	*	*	+
	C	55,000		116 ± 5.9			0
2	A	1,000			14.4 ± .37*	16.1 ± .15	+
	D	40,000				14.4 ± .15	0
	E	93,000				14.4 ± .35	0
3	B‡	600	134 ± 2.4	71 ± 2.2	12.4 ± .45	15.7 ± .70	+
	A	1,000		93 ± 5.6		14.7 ± .80	+
	F‡	36,000		129 ± 1.2		12.4 ± .99	0
	G	54,000		125 ± 2.1		12.2 ± .44	0
	E	93,000		122 ± 1.5		12.2 ± .55	0

* Values given are the means and their standard errors.
† Determined by turbidity-reducing method of Dorfman and Ott(8).
‡ These groups contained 4 animals, all other groups consisted of 5 rats each.

local injection of active material, and (2) a study of the appearance of edema, change in hemoglobin concentration and rate of disappearance of a colloidal dye from the blood following intravenous administration of enzyme preparations. Evidence is presented to show that purified hyaluronidase does not have vascular permeability-increasing activity.

Methods and materials. Female Sprague-Dawley rats weighing approximately 195 g were fed Purina chow and water *ad lib.* Testis hyaluronidase preparations were administered intravenously immediately following an injection of Evans blue, as previously described(5). Weights were taken at the time of injection. Thirty minutes after the administration of enzyme the concentration of the dye in the blood was determined and the animals were examined grossly for evidence of edema and dye in the 4 paws and snout. In some instances, hemoglobin concentration was measured.

Extracts of bull testis containing hyaluronidase activity† were fractionated with alcohol in the cold under controlled conditions of pH and ionic strength(7). Enzyme thus prepared varied in purity from 36,000 to 93,000 units per mg N. Approximately equivalent quantities of various preparations, in terms of hyaluronidase activity (2000 units), were administered and their effects on vascular permeability were compared with those of two crude preparations of enzyme; A, containing 1000 units per mg N and B, having 600 units per mg N. The latter preparation served as starting material for the more purified extracts (F, G, and E, see Table I). Hyaluronidase activity was determined by a turbidity reducing method, the unit being related to an arbitrary standard(8).

The effect of local injection of 2 extracts was tested in 5 rats. Following the intravenous administration of 2 mg of Evans blue, 0.05 ml of 0.85% saline containing 25, 2.5 and 0.25 units of preparation A was injected intracutaneously in the shaved skin of the back. Parallel injections of similar quantities of the purified enzyme, D, were made in the same animals. Five hundredths ml of 0.85% saline was injected intracutaneously in each rat as a control. Observations were made on the extravasation of dye around the injection sites 15 minutes following the administration of enzyme.

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† Supplied through the courtesy of the Armour Laboratories.

7. Freeman, M. E., Anderson, P., Webster, M. E., and Dorfman, A., *J. Biol. Chem.*, 1950, v186, 201.

8. Dorfman, A., and Ott, M. L., *J. Biol. Chem.*, 1948, v172, 367.

Results. The data summarized in Table I demonstrate that each hyaluronidase preparation was administered in approximately equivalent quantities in terms of enzyme activity. They also demonstrate that the crudest enzyme preparations (600 and 1000 units per mg N), prepared essentially by the method of Claude and Duran-Reynals(9), had vascular permeability-increasing activity as manifested by the grossly apparent edema and tissue coloration, the increased hemoglobin concentration and/or dye disappearance. In sharp contrast, is the lack of such effects when enzyme preparations of substantially higher purity were tested (36,000 to 93,000 per mg N).

Local extravasation of dye in excess of the saline control was induced by the relatively crude testis extract, A, in quantities of 25 and 2.5 units, but not when 0.25 unit of hyaluronidase activity was administered. With the purified enzyme, D, only very slight evidence of increased dye extravasation was obtained when 25 units were administered locally. No increased loss of dye was seen with this preparation administered in smaller amounts.

Discussion. Relatively crude preparations of testicular hyaluronidase, prepared by modifications of the method of Claude and Duran-Reynals(9), have been used in the majority of previous investigations(1-5). It is not surprising, therefore, that similar effects on vascular permeability were obtained. Recently, Elster *et al.*(10) described testing a more highly purified preparation (35,000 units per mg N) in which vascular permeability-increasing activity was found. They used 3 to 4 times the amount of hyaluronidase we have used and noted that even with this quantity of enzyme the effect on vascular permeability was less prolonged than that produced by the cruder preparations suggesting that there may have been some reduction of the permeability-increasing activity in their material.

It has been reported that preparations having hyaluronidase activity showed no evidence of inducing increased vascular permeability. Zweifach and Chambers(11), using direct observation of mesenteric capillaries, did not observe permeability increases short of actual rupture of the capillary wall. Local injection of hyaluronidase-containing extracts into the skin of rabbits did not produce any leakage of intravascular dye in the hands of Rocha e Silva and Dragstedt(12). Both groups of investigators used preparations obtained from Dr. Karl Meyer at Columbia University. Danielli and Stock(13) also make mention of the fact that in their frog leg perfusion preparations they could find no evidence of vascular permeability increase but only of vasodilator activity. The source of their preparation is not mentioned.

The results reported in this paper indicate clearly that purification of the hyaluronic acid depolymerase present in testicular extract does not lead to purification of the factor capable of increasing vascular permeability. On the contrary, the vascular permeability-increasing activity disappears with purification of the enzyme. The capacity to increase vascular permeability resident in crude testicular extracts does not, therefore, appear to be due to its content of hyaluronidase.

Preliminary qualitative observations of the spreading of India ink and Evans blue in the skin of rabbits and rats indicate that purified hyaluronidase preparations retain the spreading effects despite loss of vascular permeability-increasing activity.

We have as yet little information on the chemical nature of the vascular permeability-increasing factor. The following facts are at hand and of some interest. It is extractable, at least in part, with 0.1 N acetic acid. At least a part of it is soluble in 0.3 saturated ammonium sulfate and precipitated by 0.7 saturated ammonium sulfate. It is retained by the dialysing membranes ordinarily in

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13. Danielli, J. F., and Stock, A., *Biol. Rev.*, 1944, v19, 81.

use. Heating at 70°C for 30 minutes destroys the activity. Work is in progress to purify and further characterize the substance in the testis extract capable of increasing vascular permeability.

Summary and conclusions. The intravenous or local administration of relatively crude bull testis extract is capable of causing increased vascular permeability in rats. Purification of the hyaluronidase component of this extract results in loss of its capacity to induce increased vascular permeability. The purified hyaluronidase appears to retain its spreading activity.

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Failure of Testosterone to Alter the Responsiveness to Insulin in Diabetes Mellitus.* (18878)

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In previous studies(1,2) it was shown that the administration of desoxycorticosterone acetate to patients with diabetes mellitus altered the responsiveness to insulin. Patients who were initially insensitive were almost invariably made transiently more sensitive, whereas those who were initially sensitive became less sensitive or were unchanged. It was postulated(2) that the observed increase in sensitivity may have been due to the inhibition of contra-insulin factors from the anterior pituitary or adrenal glands. Cheng and Sayers(3) have postulated that the action of DCA in increasing sensitivity to insulin in the rat was due to the decrease in the output of 11-oxygenated adrenal steroids, secondary to the inhibition of ACTH release by the hypophysis.

The present studies are part of a series to evaluate the relative effectiveness of various steroids in altering insulin sensitivity in humans, and to determine if possible the chemical groupings associated with this phenomenon.

Procedure. Thirteen patients with well-controlled diabetes were studied. Insulin-sensitivity was determined by the Himsworth(4)-glucose-insulin tolerance test as previously described(2). After the determination of the initial sensitivity, testosterone propionate in doses indicated in Table I, was given intramuscularly for a period of 10 days, and the insulin sensitivity again determined.

Results. The initial and final values for the glucose-insulin areas are shown in Table I, and the glucose-insulin tolerance curves in Fig. 1. As has been previously described(2),

TABLE I. Glucose Insulin Areas Before and After Administration of Testosterone for 10 Days.

Daily dose of testosterone, mg	Patient	Glucose-insulin areas in mg min Before	After
10	W. M.	+ 7400	+6600
	E. T.	— 1800	— 924
	J. K.	— 140	+ 180
	J. A.	+ 1760	—1730
25	M. W.	+ 1240	—1900
	I. S.	+12,550	+5100
	E. R.	+ 3750	+4830
	W. K.	+ 2220	+2420
50	J. H.	+ 4300	+2650
	C. M.	— 650	+2340
	W. S.	+ 920	+1100
	G. P.	+ 1130	+2010
	W. L.	+ 900	+1310

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