on the anterior pole of the nucleus (Fig. 1, D, E, G); secondly, the abnormality may set in during the midspermatid stage when its topography is disturbed mechanically by the changing nucleus (Fig. 1, H, I, J, K). It was also noted that in some of the apparently normal looking spermatozoa, the acrosome, while it had the typical sickle shape, had one, and rarely two, vacuoles (Fig. 2, P, Q, S) comparable to those of human spermatozoa(8) and those of the bull(9). While these vacuoles were rarely seen, and then with difficulty, using ordinary technics, P.-A.S. technic made it possible to count them in sections of testis.

Conclusions. Most of the sperms in the sterile individuals of these genotypes were normal looking and presumably physiologically inefficient. I agree with Chang(10) in stating that a morphologically normal sperm may be physiologically incapable of effecting fertilization. The fact that the acrosome alterations as revealed by P.-A.S. technic may easily be overlooked when treated by usual methods suggests that differences in such non-chromosomal elements may be of considerable importance in evaluating morphological normality of a spermatozoon.

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Effect of Cortisone on Histamine Liberation Induced by Tween in the Dog.* (19239)

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The inhibitory effect of cortisone on certain experimental hypersensitivity reactions has been shown by several investigators. Anaphylaxis in the mouse(1,2), the Arthus phenomenon in the rabbit(3,4), the tuberculin reaction in the guinea pig(5,6), and the Shwartzman reaction in the rabbit(7) represent hypersensitivity reactions which are influenced by cortisone. In addition, clinical studies too numerous to mention indicate that improvement may follow the administration of cortisone in a variety of human diseases in which hypersensitivity appears to play a part. Somewhat paradoxically, anaphylactic shock cannot be prevented in the guinea pig(8,9) with cortisone administration, although certain features of passive anaphylaxis in this species may be modified *in vitro*(10). Also, passive anaphylaxis in the dog(11) is uninfluenced by

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cortisone treatment. Despite extensive investigations, the mechanism of action of cortisone in hypersensitivity has not been established. From the available evidence, it is not clear whether the important action of cortisone is exerted on the immune mechanisms involved or on the liberation and effect of pharmacologically active compounds which may be released by antigen-antibody reactions.

In view of the lack of understanding of the mode of action of cortisone in hypersensitivity, it seemed worthwhile to study the possible effect of this drug on the reaction induced by Polyoxyethylene derivative of sorbitan monolaureate (Tween 20) in the dog. This remarkable phenomenon, first reported by Krantz and coworkers(12,13), shows many similarities with anaphylaxis in the dog, including the liberation of a histamine-like substance. It is, however, a simpler phenomenon than anaphylaxis, since no antibodies or antigen-antibody reactions are involved. Because of the relative simplicity of the Tween phenomenon, it seemed likely that any effect of cortisone on it would be more subject to analysis in terms of mechanisms involved than the effect of cortisone on anaphylactic hypersensitivity. The data which form the basis of this report indicate that the compound liberated in the dog by Tween 20 is histamine, and they offer suggestive evidence for the ability of cortisone to influence histamine metabolism.

Material and methods. Male dogs were used. Two injections of Tween 20† were given intravenously at either 24 or 48 hour intervals under pentobarbital anesthesia. The dose of Tween 20 was 0.1 ml per kg of a 5% solution in water. Arterial blood pressure was recorded from the femoral artery with a mercury manometer. Ten dogs received 4-7 mg of cortisone‡ per kg of body weight intramuscularly every 6 hours day and night. Cortisone treatment was begun 6 hours prior to the first Tween injection and was continued throughout the experiment for 24 or 48 hours. Eleven dogs received the Tween injections without cortisone treatment and served as controls. Histamine in the plasma was demonstrated by paper chromatography. The method described by Urbach(14) with the extraction procedure of McIntire(15) was used with the modification of employing ascending instead of descending chromatography. Blood was drawn immediately before and 5 minutes after the injection of Tween. Citrate was the anticoagulant used. Twenty ml of plasma was the usual volume for the extraction of histamine. Known quantities of histamine were added to dog's plasma. These plasma samples were extracted simultaneously with the unknowns and the final butanol extracts were chromatographed on the same sheet of filter paper.

Results. A. Demonstration of histamine in dog's plasma following the injection of Tween 20. When dog's plasma obtained 5 minutes after the injection of Tween 20 was analyzed for histamine by paper chromatography, it produced a spot of the same Rf value and the same color as plasma samples to which known amounts of histamine were added. This fact has been demonstrated conclusively in 8 separate experiments. The paper chromatographic technic is not suitable for the exact quantitation of the amount of histamine released by Tween 20. Nevertheless, certain conclusions may be drawn from these studies. In some experiments, in which a 25% solution of Tween 20 was injected intravenously, the amount of histamine found in 20 ml of plasma was greater than 10 μg. In the majority of the experiments, the quantity of histamine was estimated to lie between 5 and 10 μg per 20 ml of plasma.

B. Effect of cortisone on the Tween reaction. Table I shows the effect of 2 injections of Tween 20 on the mean blood pressure of male dogs. The first injection produced a marked fall of blood pressure. The second injection, given 24 or 48 hours later, produced a lesser but still significant fall of blood pressure in all untreated control animals. Cortisone had no effect on the response to the first Tween injection. It decreased or prevented the response to the second injection. The differences between cortisone treated and untreated male dogs appear to be striking

† Tween 20 was obtained from the Atlas Powder Co., Wilmington, Del.

‡ Cortone Acetate Merck.

TABLE I. Blood Pressure Changes in Cortisone-Treated and Untreated Male Dogs Following Two I.V. Injections of Tween 20.

Dog No.	Treatment	Intervals, hr	Tween injections: First (mm of Hg) Initial	First 5 min	First 10 min	Second (mm of Hg) Initial	Second 5 min	Second 10 min
1	None	24	130	42	42	125	98	75
2			125	41	42	132	92	86
3			122	42	33	110	65	42
4			115	42	40	110	85	96
5			115	41	42	119	87	82
6			110	50	52	108	62	52
7			108	48	63	92	47	52
		Mean ± S.D.	118 (8.1)	44 (3.7)	45 (9.7)	114 (13)	77 (18.7)	69 (20.1)
8	None	48	130	48	32	122	70	80
9			125	50	61	92	60	73
10			120	50	51	128	75	90
11			115	60	65	115	95	106
		Mean ± S.D.	122 (6.5)	52 (5.4)	52 (14.7)	114 (15.8)	75 (14.7)	87 (14.3)
12	Cortisone	24	150	55	50	100	60	84
13			144	65	64	110	127	98
14			123	45	45	126	115	124
15			120	50	52	120	105	115
16			120	50	56	95	96	98
17			120	43	47	114	82	70
18			105	50	57	90	90	89
		Mean ± S.D.	126 (15.6)	51 (7.3)	53 (6.5)	108 (12.5)	96 (22.1)	97 (18.3)
19	Cortisone	48	162	58	57	140	140	138
20			138	86	62	120	141	150
21			130	58	75	115	119	119
22			120	56	70	110	118	130
		Mean ± S.D.	137 (17.9)	64 (14.4)	66 (8)	121 (13.2)	129 (12.7)	134 (13.1)

when the second injection of Tween 20 was administered 48 hours after the first injection. Under these conditions, the 4 cortisone treated dogs were completely refractory to the hypotensive action of the second injection of Tween 20. In 2 of these dogs the blood pressure actually rose.

In addition to these experiments, 2 male dogs received cortisone treatment in the usual way for 24 hours before receiving the injection of Tween 20. Cortisone again failed to prevent the usual response of dogs to the first injection of Tween.

Three of the dogs which were refractory to the second injection of Tween 20 as a result of treatment with cortisone received an intravenous injection of histamine (10 μg per kg). They responded with a marked fall of blood pressure to the injection of histamine. Fig. 1 shows the blood pressure tracing of one of these experiments. The cortisone treated dog responded to the injection of histamine, whereas it failed to respond to the second injection of Tween 20.

A limited number of experiments were performed on female dogs. Cortisone treatment produced complete refractoriness to the second injection of Tween 20 in only one out of 5 female dogs. These experiments suggest that it may be more difficult to demonstrate the interference of cortisone with the Tween phenomenon in female dogs.

Discussion. Since our paper chromatographic studies show that Tween 20 liberates histamine in the dog, the interference of cortisone with the hypotensive effect of a second injection of Tween 20 is probably related to an effect of cortisone on the production, destruction or release of histamine. It is obvious that cortisone does not interfere with the release of preformed histamine, since it does not protect against the first injection of Tween 20. It is also obvious that cortisone does not protect against the effects of re-

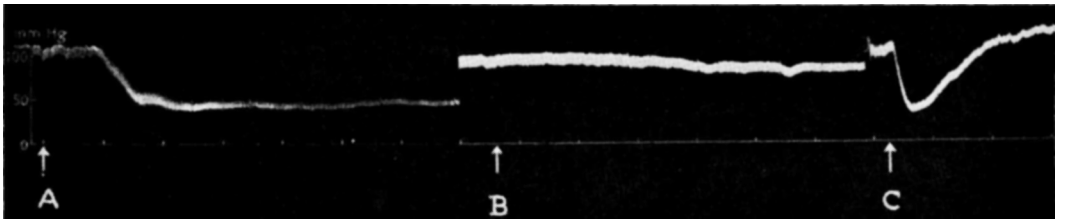

FIG. 1. Effect of inj. of Tween 20 and of histamine on mean blood pressure of a dog treated with cortisone. At A, Tween 20 was inj. At B, inj. was repeated 24 hr after the first inj. At C, histamine was inj. Time in min.

leased histamine. The most likely possibilities are that cortisone prevents the resynthesis of histamine in the tissues following its release and/or it stimulates the destruction of histamine in the tissues. Either one of these hypotheses would explain why the second injection of Tween 20 fails to produce hypotension.

The effect of cortisone on Tween hypersensitivity may be looked upon as a prolongation of the refractory period which according to Krantz and coworkers(12) lasts 14 to 16 hours. Unfortunately the nature of the refractory period is not well understood. Krantz suggests that the refractory period represents the time required for the destruction of Tween. If this hypothesis were substantiated by experimental data, it could be argued that cortisone influences the Tween phenomenon by delaying the destruction of the compound.

There are no data in the literature concerning the breakdown of Tween 20 in the dog. Experiments on the destruction of this compound by isolated rat tissues(16) indicate that Tween 20 is broken down by active esterases which are present in most tissues. It is difficult to believe that cortisone could prevent the destruction or excretion of Tween 20 for 48 hours in the dog, and it is very unlikely that our data could be explained on that basis. The failure of cortisone to prevent anaphylaxis in the guinea pig, where histamine release is quite certain, is comparable with the failure of cortisone to prevent the response of dogs to the first injection of Tween. It should be of interest to study the effect of cortisone on repeated anaphylactic shocks in the guinea pig and such experiments are in progress.

The results of this study fit in best with the hypothesis that cortisone is capable of influencing histamine metabolism in the dog in such a way that it prevents the accumulation of histamine in the tissues once they are depleted of that compound by the action of Tween 20. Only further studies will show whether or not the observations and interpretations of the present study may contribute to the understanding of the mechanism of action of cortisone in hypersensitive states.

Conclusions. (1) The injection of Tween 20 in the dog is followed by the appearance of histamine in the plasma, as demonstrated by paper chromatography. (2) Cortisone does not prevent the hypotensive effect of the first injection of Tween 20, in the male dog. (3) Cortisone prevents or decreases the hypotensive effect of a second injection of Tween 20 administered 24 or 48 hours after the first injection. (4) Cortisone does not prevent the hypotensive effect of injected histamine. (5) It is postulated that cortisone prevents the accumulation of histamine in the tissues following the release of this compound by Tween 20.

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A Hitherto Unrecognized Factor Against Dietary Necrotic Liver Degeneration in American Yeast (Factor 3) (19240)

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Most American yeasts, in contrast to many European yeasts, do not induce necrotic liver degeneration when used as protein source in purified, vit. E free rations for rats. However, the deficiency can be obtained readily with American grown torula yeast(1), and it can be prevented either by cystine or by vit. E (2). In earlier work some evidence had been accumulated for the involvement of a third unidentified factor in the prevention of the defect(3). It had been noted particularly that the addition of a few percent crude casein, or ordinary "vitamin free", alcohol extracted casein to necrosis producing diets inhibited the damage,* whereas purified, alkali-treated casein "Hammarsten" did not influence the development and had even been used extensively as a main dietary component for the production of the deficiency(4).

It is shown in the following experiments that American yeasts G† and K.‡ which do not produce the defect(1) have a considerable protective activity when added in small amounts to torula diets. It is concluded from simple fractionation experiments with yeast K that this effect is not due to the presence of vit. E or cystine but that protection is brought about by a third, unidentified principle which has been designated "factor 3."

Experimental. Details concerning the induction of the deficiency have been described (1). Weanling Sprague-Dawley rats of the National Institutes of Health strain were used exclusively. Animals in this series were males except for a few groups of mixed sex as indicated. Each group started with 10 animals. Groups in one experiment consisted of equally distributed litter-mates of even average weight. The basal ration, as reported previously, contained 30% torula yeast, 59% sucrose, 5% salts, 5% vit. free lard and a vitamin mixture(1). From this formula the different experimental diets were derived by adding supplements at the expense of torula or of sucrose. Tests for protective activity were performed in two ways: (A) Prophylactic method (Table I, exp. A, B and C). The supplement was given from the beginning of the experiment. (B) Repletion method (Table II). The animals were kept on the basal diet, in sets of 5 litter mates to a cage, for a preliminary period of 23 days. At the 24th day they were separated and supplementation was started. The repletion method was adopted as a standard procedure, since it requires less space and less supplemented material and yields results in a shorter experimental time. It is based on two observations: Very few rats succumb to dietary necrotic

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† Cultured, primary dried yeast, Anheuser-Busch, Inc., St. Louis, Mo.

‡ Debittered, dried brewer's yeast, Anheuser-Busch, Inc.