solution of known volume. The optimum mixture contains 60–80% acetone or dioxane, the exact proportion depending upon the relative amounts of fat and inorganic salts in the sample. If more than one dilution is made, it is economical to use acetone for the first dilution. However, because of its higher boiling point and density, dioxane[5] is to be preferred for the final dilution if the sample is to be counted as a liquid. In a few instances the mixing of acetone with concentrated nitric acid solutions was followed by evolution of heat and boiling of the acetone, which caused loss of sample. This difficulty was not encountered when dioxane was used for all the dilutions.

Using the above method we have been able to obtain good results with Au^{198} as well as with Hf^{181}. We were able to recover approximately 95% of radioactive colloidal gold added to a whole rat and to a dog liver before digestion. In another experiment the total activity found in a whole dog liver by this method differed less than 3% from the total activity found by counting the aqueous and fatty layers separately.

The use of acetone or dioxane to make a homogeneous nitric acid tissue digest is recommended as a more accurate and simple method for the determination of the isotope content of animal tissues.

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[5] Dioxane should be handled with due precautions in view of its inflammability, the toxicity of its vapors, and the possible presence of explosive peroxides.

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Effect of Ascorbic Acid on the Adrenal Weight of Normal and Hypophysectomized Rats[1]

Louis-Paul Dugal and Mercedes Thérien[2]

Département de Physiologie expérimentale, Faculté de Médecine, Université Laval, Québec, Canada

Ascorbic acid, in large doses, has been reported to increase the fall in adrenal cholesterol and to have other ACTH-like effects (*1–3*). It has also been shown that the same substance prevents the normal hypertrophy of the adrenals in animals exposed to cold (*4*), as well as the alarm reaction in the same conditions of exposure (*1*) or under epinephrine treatment (*5*). In the case of exposure to cold, ascorbic acid also increased the survival of the animals (*4*).

The purpose of the present investigation was to try to clarify and correlate the above findings. At least three working hypotheses might explain why ascorbic acid prevented the normal hypertrophy of the adrenals during stress: (1) ascorbic acid may inhibit directly the action of ACTH on the adrenal, although this seemed rather unlikely; (2) it has a corticotrophic effect; the hypersecretion of cortical hormones thus produced (an assertion substantiated by the fact that the fall in adrenal cholesterol is greater with, than without, ascorbic acid during exposure to cold [*1*]) would prevent, indirectly, the hypersecretion of ACTH, according to Sayer's theory (*6*); or (3) that ascorbic acid has a potentiating action on ACTH when the demand for cortical hormones increases. If the last possibility is correct, one would expect that in a case of stress the hypophysis would not be hyperstimulated by the lack of cortical hormones, which are produced in sufficient amount by the combined action of small doses (approximately corresponding to normal secretion) of ACTH and ascorbic acid.

Consequently, three series of experiments were performed. In the first, we wanted to learn whether ascorbic acid prevents the action of injected ACTH on the adrenals of normal rats. Sixty rats, averaging 200 g in weight, and fed Purina Fox-Chow *ad lib.*, were divided into 4 groups: One group (A) received by daily intraperitoneal injection, during 3 days, 150 mg of sodium ascorbate; a second group (B) received the same amount of sodium ascorbate plus 10 mg daily of ACTH by intramuscular injection; a third group (C) received sodium bicarbonate intraperitoneally, the amount of sodium injected daily being equivalent to that injected into the first group; and the last group (D) received sodium bicarbonate intraperitoneally plus 10 mg/day of ACTH by intramuscular injections. The results, presented in Table 1, show that ascorbic acid does not prevent the action of injected ACTH. The increases in adrenal weight in the ascorbate and bicarbonate groups are both statistically significant and do not differ from one another.

The second and third series of experiments were done on hypophysectomized rats, fed the special diet of Shaw and Greep (*7*). In each case, the left adrenal was removed 10 days after hypophysectomy and before any treatment. Comparisons were then made on each individual between the right adrenal after treatment, and the left one before treatment. Table 2 shows that ascorbic acid has no effect on the adrenal weight of hypophysectomized animals, at least for the 10-day period of treatment we have used.

In the third series of experiments, we tried the effect of a small dose of ACTH (0.5 mg/day) combined with ascorbic acid. Both were given by intraperitoneal injection. The dose of ACTH employed had no effect by itself on the adrenal weight, nor did the combination of ACTH (same dose) and bicarbonate (solution adjusted so as to inject the same amount of sodium daily into this group as into the sodium ascorbate group) have any effect; but the combination of the same dose of ACTH with ascorbic acid (150 mg/day) had a pronounced and significant effect. Whereas there is a small decrease in the first two groups (Table 3) between the weights of the right adrenal after treatment as indicated and the left adrenal before treatment, there is, on the contrary, an

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[2] Research fellow from the Canadian Life Insurance Officers Association.

TABLE 1

INFLUENCE OF ACTH ALONE AND OF ACTH COMBINED WITH ASCORBIC ACID ON THE ADRENAL WEIGHT OF NORMAL RATS

Group	No. of animals	Treatment	Days of treatment	Adrenal wt* (mg)	Diff (%)	t
A	14	150 mg daily of sodium ascorbate intraperitoneally	3	34.94 ± 1.19†		
B	16	150 mg daily of sodium ascorbate intraperitoneally + 10 mg daily ACTH intramuscularly	3	40.24 ± 1.30	15.2	3.01
C	14	$NaHCO_3$ intraperitoneally; Na equivalent to A	3	35.21 ± 1.38		
D	16	$NaHCO_3$ intraperitoneally and 10 mg ACTH daily intramuscularly	3	41.03 ± 1.33	16.5	3.04

* Fresh weights.
† Standard error.

TABLE 2

EFFECTS OF ASCORBIC ACID ON THE WEIGHT OF THE RIGHT ADRENAL OF THE HYPOPHYSECTOMIZED RAT

Treatment	Duration of the experiment after hypophysectomy (days)	Days of treatment	No. of animals	Adrenal wt* (mg)		Diff (%)
				Left	Right	
$NaHCO_3$ Na equivalent to the other group, intraperitoneal injections	20	10	19	5.89	6.36	7.9
150 mg daily of Na ascorbate, intraperitoneal injections	20	10	19	5.94	5.98	0.6

* Fresh weights.

important and very significant increase in adrenal weight (right compared to left; increase of 25%, $t = 3.61$) in those hypophysectomized animals receiving daily for 10 days, 0.5 mg ACTH and 150 mg ascorbic acid, in the form of sodium ascorbate.

That the sodium had nothing to do with the phenomenon is clear from the fact that controls receiving sodium bicarbonate plus the same amount of

TABLE 3

INFLUENCE OF ASCORBIC ACID COMBINED WITH ACTH AND OF ACTH ALONE ON THE ADRENAL WEIGHT OF THE HYPOPHYSECTOMIZED RAT

Treatment	Duration of experiment after hypophysectomy (days)	Days of treatment	No. of animals	Adrenal wt* (mg)		Absolute diff (mg)	Diff (%) between right and left adrenal
				Left	Right		
ACTH, 0.5 mg/day intraperitoneally	25	10	19	5.99 ± 0.32†	5.73 ± 0.42	− 0.26	
$NaHCO_3$ + ACTH, 0.5 mg/day intraperitoneally	25	10	10	5.53 ± 0.23	5.28 ± 0.25	− 0.25	
Na Ascorbate, 150 mg/day + ACTH, 0.5 mg/day intraperitoneally	25	10	21	5.79 ± 0.20	7.27 ± 0.36	+ 1.48	+ 25.6 ($t = 3.61$)
Diff (%) between weight of right adrenals					Between 1 and 3 26.9% ($t = 2.79$)	Between 2 and 3 37.7% ($t = 4.54$)	

* Fresh weights.
† Standard error.

ACTH showed a slight decrease in weight of the right as compared to the left adrenal, instead of a significant increase.

Histological studies were also made on the same adrenals and will be published separately (*8*). Let it suffice to say here that, as far as the fall in cholesterol and the measures (with a planimeter) of the different zones of the adrenal cortex are concerned, the results parallel exactly the ones that we have just described for the adrenal weight.

Such a result may mean that a *normal* secretion of ACTH, plus large doses of ascorbic acid, has the same effects as much larger doses of ACTH alone, when the doses correspond to hypersecretions in normal animals submitted to stress.

The fact, just described, that ascorbic acid synergizes the action of ACTH, at least in hypophysectomized animals, seems to contradict the alternative that ascorbic acid prevents the hypertrophy of the adrenals in a case of stress. But the conditions of both experiments were not the same, and, moreover, we do not know yet if the potentiating action of ACTH by ascorbic acid is quantitatively the same, whether we consider the effect on adrenal weight (AWF) or on the cholesterol fall of the adrenals. In other words, it is possible that small doses of ACTH plus ascorbic acid, which would have the same effects on the fall of adrenal cholesterol as larger doses of ACTH alone, would have a slower or less intense effect than the same doses of ACTH alone on the adrenal weight.

One might also wonder why there is such a great difference in the dosage of ACTH between the first and the third series of experiments. The reason is that, in the first series, we wanted to learn whether ascorbic acid prevented the action of ACTH, when ACTH was actually present, and we wanted to be sure to obtain the action of ACTH within 3 days; but we did not use the same dosage of ACTH for the third series, because there we wanted to show that a small dosage of ACTH would have a stimulating effect on the adrenal weight only if combined with ascorbic acid, a result which would necessarily have been masked by large dosages of ACTH.

From these experiments it may be concluded that (1) in normal rats, ascorbic acid does not prevent the action of injected ACTH on the adrenal weight; and (2) in hypophysectomized rats, ascorbic acid alone has no effect on the regeneration of the adrenal cortex but potentiates the effects of ACTH on the same process.

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The Interaction of Hyaluronidase with Thromboplastic Components of Blood Coagulation

Silvio Fiala, D. R. Meranze, and Karl Roth

Department of Research, Southern Division, Albert Einstein Medical Center, Philadelphia, Pennsylvania

Although it is known that the activity of hyaluronidase may be inhibited by normal mammalian sera (*1*), no attention seems to have been paid so far to the interaction of hyaluronidase with the clotting mechanism of plasma. During investigations of the first stage of the blood clotting process (activation of prothrombin) it was noted that the plasma thromboplastic material is affected by dialyzed bovine hyaluronidase. Fig. 1 summarizes the observations showing the clot-inhibitory effect of hyaluronidase on human oxalated plasma after 10 min incubation at 38° C. Enzyme heated to 60° for 15 min has no activity. Incubation of plasma with the enzyme did not affect the prothrombin time (Quick's one-stage) indicating that prothrombin, labile factor (Ac-Globulin), and fibrinogen were not attacked by the hyaluronidase. This restoration of the coagulability of the incubated plasma-enzyme mixture suggested that the enzyme was acting against a thromboplastic component. In agreement with this, it was found that the inactivating effect of the enzyme could also be corrected by the addition of washed, isolated, human platelets. If, however, the platelets were first incubated with

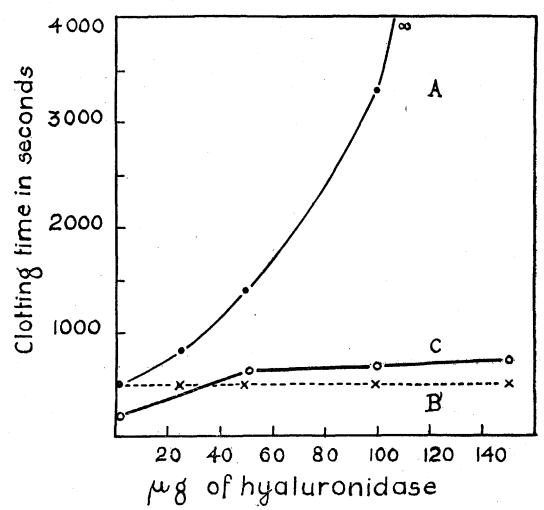

FIG. 1. The effect of dialyzed (48 hr, against saline) bovine hyaluronidase (Armour) on the clotting time of human oxalated plasma. Plasma-enzyme mixture incubated at 38° C for 10 min; pH, 7.5. After incubation, plasma recalcified with 0.25 *M* $CaCl_2$. Curve *A*, plasma incubated with active enzyme; Curve *B*, incubated with enzyme heated to 60° C for 15 min; Curve *C*, after incubation of plasma with enzyme, 0.1 ml isolated, washed, human platelets (5×10^5 mm³) were added to 1.0 ml of the incubated mixture. The prothrombin time of the unincubated control was 14.6 sec. Addition of 0.2 ml rabbit brain thromboplastin (acetone-dried) to all incubated recalcified plasma-enzyme mixtures restored coagulation in 14.8 sec.