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

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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

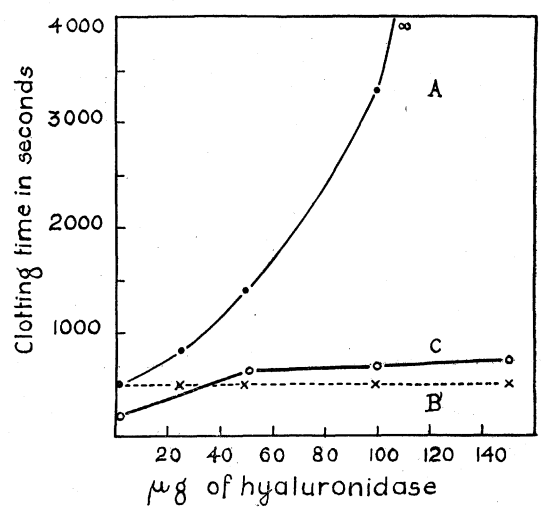

FIG. 1. The effect of dialyzed (48 hr, against saline) bovine hyaluronidase (Armour) on the clotting time of human oxalated plasma. Plasma-enzyme mixture incubated at 38° C for 10 min; pH, 7.5. After incubation, plasma recalcified with 0.25 *M* $CaCl_2$. Curve *A*, plasma incubated with active enzyme; 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.

TABLE 1

PROTECTION OF HUMAN PLASMA AGAINST THE EFFECT OF HYALURONIDASE BY DIFFERENT CONCENTRATIONS OF PLATELETS*

Amount of platelets (in mm^3 of added suspension)	0	1×10^5	2.5×10^5	5×10^5	2×10^6
Clotting time of platelet-plasma mixture	177 sec	94 sec	85 sec	85 sec	82 sec
Clotting time of platelets-hyaluronidase-plasma mixture	∞	$>$ 1000 "	820 "	450 "	197 "
Prothrombin time of platelets-hyaluronidase-plasma mixture (after addition of 0.2 ml brain thromboplastin)	10.1 sec	10.2 "			10.1 "

* Five tenths ml of platelet suspension incubated for 30 min at 38° C with 1 mg dialyzed bovine hyaluronidase. After incubation, 0.5 ml human oxalated, dialyzed plasma was added to each sample and incubated for 10 min. Recalcified with 0.5 ml $CaCl_2$ (0.02 *M*), pH 7.5.

hyaluronidase, their clot-accelerating activity was overcome and the enzyme retained less activity (or none) against the plasma (Table 1). The enzyme, counteracted by an excess of tissue (brain) thromboplastin, could inhibit the diluted thromboplastin (1:100–1:1000), so that in respect to hyaluronidase inhibition the same factor appears present in both tissue and platelet thromboplastic component.

The enzyme after its incubation with plasma could not be recovered. There is, moreover, some evidence that the effect of clotting inhibition by even small amounts of hyaluronidase is not dependent on the incubation time. Thus it seems likely that, rather than providing a substrate in the conventional sense, the thromboplastic component both in plasma (from the platelet material still present) and in platelets interact with the enzyme competitively, with resulting inhibition of the plasma clotting mechanism and of hyaluronidase, one or the other inhibition being more in the foreground, depending upon the relative excess of either hyaluronidase or platelet material.

So far as platelets are concerned, it was found that these elements do not contain any measurable amount of nucleic acid (UV-spectra of sonically disrupted platelets) but, in addition to protein material absorbing at 280 mμ and inactive in coagulation, they do contain material that competes with hyaluronidase, withstands boiling for 15 min, and is not precipitated by 5% trichloracetic acid. This nonprotein material which does not pass through the cellophane dialysis membrane has the same accelerating effect on the coagulation as the intact platelets. The chemical nature of this thermostable, nonprotein thromboplastic material, which interacts with hyaluronidase in the same manner as described above, and which corresponds to what has been designated by Howell (*2*) as "cephalin" thromboplastic factor, is under investigation by means of paper chromatography. In connection with the inhibitory effect of hyaluronidase on thromboplastic blood component the further purification of enzyme preparations is needed. It may be recalled, also, that the thromboplastic effect of highly polymerized hyaluronic acid has been reported (*3*).

Although the concept of the interaction of platelets and a plasmatic factor in constituting the natural thromboplastin in blood coagulation is fully valid, the particular formulation of Quick (*4*), supposing the active principle in platelets to be an enzyme (thromboplastinogenase), is incompatible with the findings here reported, which exclude the protein nature of the thromboplastic platelet factor. A detailed study of the thromboplastic system operating in blood will appear shortly.

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A Tissue Chamber and Splint for the Mouse[1]

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Technical problems of splint and chamber construction encountered while engaged in tissue chamber studies of skin grafts in mice prompted some experimentation. A type of splint was devised which could be constructed from copper wire, sheet lucite, and solder with the aid of a few tools. It is offered to those who wish to undertake this fundamental mode of animal experimentation, but who have little machine-shop aid, and whose laboratory time is limited. It is well tolerated (4 weeks or longer) and satisfies the basic requirements of (1) adequate immobilization and protection, (2) maintenance of proper circulation within the chamber, and (3) provision for satisfactory microscopic observation. It has been used successfully in conducting several series of studies of skin grafts in mice.

Although the essential principles of construction described in detail by Algire and Legallais (*1*) have been followed, the skin flap is immobilized with a traction splint of copper wire, and the tissue chamber proper is incorporated within one of the lateral splints of lucite. The chamber itself is closely similar to the

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