Work is in progress to determine the smallest biologically active moiety in urine, its chemical nature, and whether its primary site of action is bone marrow or some other tissue or organ.

Summary. Urinary erythropoietic factor has been partially purified by adsorption on kaolin followed by serial elution with ammonium acetate buffers of increasing pH and then with 1M NH_4OH. Using this procedure, a 39% yield with a 230 fold purification of the active factor has been obtained. The purified preparation of the active factor produces metachromasia with toluidine blue, has an absorption peak at 280 mμ, and migrates very slowly on paper strips in an electrophoretic apparatus. The similarity and differences between urinary and plasma erythropoietin are discussed.

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Influence of Cortisone, Medrol, and Aristocort Diacetate on Antitryptic Activity of Rat Serum. (24042)

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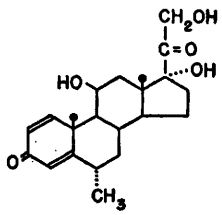
The view has been expressed that an intimate physiological relationship exists within the circulating blood between fibrin formation and dissolution (fibrinolytic) mechanisms and the integrity of the blood vessel wall(1,2). Previous work(3,4) has presented evidence that the pituitary-adrenal cortex axis exerts a controlling influence on the fibrinolytic (plasmin-antiplasmin) system in the blood. In continuation of studies(5) on the influence of certain modified corticosteroids, more potent than cortisone, on antitryptic activity of rat serum, Medrol (Δ^1-6-α methylcortisol) and Aristocort diacetate (16α-hydroxy-9α-fluorocortisol-16α, 21 diacetate) were compared with cortisone in normal, hypophysectomized and adrenalectomized rats.

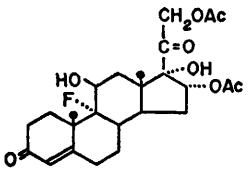
Materials and methods. Male Sprague-Dawley rats weighing 150-200 g were used. Normal and hypophysectomized animals were allowed Purina Laboratory chow and water *ad libitum,* while adrenalectomized rats were fed Purina Laboratory chow and 0.9% saline *ad libitum.* All administrations were given intramuscularly; controls received either no injections, or a volume of 0.9% saline equal to that used in the steroid injections. Adrenalectomy and hypophysectomy were performed

under ether anesthesia. Administration of the steroids was started 3 weeks after the operation. Suspensions of the following steroids were made by trituration and subsequent shaking with 0.9% saline to concentrations of 500 μg/ml and 2.0 mg/ml respectively: Cortisone, Medrol, and Aristocort diacetate.* The chemical structure and alternate nomenclature of the 2 latter steroids is listed in Fig. 1. The animals were given intramuscular injections of 1 μg or 4 μg per g of body weight at 24 hours intervals in the thigh muscles of either the left or right hind leg. These injections were continued for a period of 3 days. Blood samples of 2.0 ml were taken by heart puncture 72 hours and 10 days after the initial injection. Those animals assayed at 72 hours were injected at 0, 24, and 48 hours while in the 10 days study, the animals were injected at 0, 24 and 48 hours and kept 7 days before assay. The blood samples were allowed to clot at room temperature, centrifuged after 1-3 hours and the sera kept frozen until tested. Five or more animals were studied at each dosage level in the experimental groups. *Test for antitryptic activity.* The details of this procedure have been described(5,6). Antiproteolytic units refer to antitryptic activity of the experimental serum samples in terms of a "standard normal serum preparation" to which an arbitrary value of 100 antitryptic units per ml was assigned (5,6). All assays were performed in duplicate.

Results. The influence of the different corticosteroids at varying dosage levels on antitryptic activity of blood in the experimental groups is illustrated in Fig. 2. Each bar represents an average of 5 or more animals. Baseline values were obtained by averaging the saline and uninjected controls. This appeared justified since no differences in antitryptic activity were observed between these 2 groups. The baseline value represents an average of about 20 animals. It was found that the antitryptic activity returned to the preinjection level upon discontinuing the



MEDROL = Δ'-6 α-METHYLCORTISOL
(6 α-METHYL PREDNISOLONE)



TRIAMCINOLONE ACETATE (ARISTOCORT DIACETATE)
16 α-HYDROXY-9 α-FLUOROCORTISOL-
16 α, 21-DIACETATE

FIG. 1. Structure of steroids investigated.

steroid administration (10 days values). The serum antitryptic values of the adrenalectomized animals were below those of the normal controls, while hypophysectomy produced baseline values lower than those found after adrenalectomy (Fig. 2). In the analysis of the effect of the steroids on antitryptic serum activity, each of the experimental groups will be considered separately.

Unoperated animals. Indication of increased antitryptic activity was noted upon cortisone (1 μg/g body weight) administration, with only a slight increase when the dosage level was increased to 4 μg/g body weight. Medrol-induced responses at 1 μg were equivalent to cortisone administration at the higher dosage level. The increased antitryptic activity at 4 μg falls within the highly significant range of $p<0.01$. Aristocort diacetate produced the most marked elevation in serum antitryptic activity; increasing the dose level to 4 μg produced no further elevation.

Hypophysectomized rats. Cortisone administration at 1 μg/g body weight produced no change, but an elevation in antitryptic activity was noted at the higher dosage level. Similarly, Medrol-induced changes were apparent

* Cortisone was kindly supplied by Merck and Co., Rahway, N. J.; Medrol by The Upjohn Co., Kalamazoo, Mich., Aristocort diacetate by the American Cyanamid Co., Pearl River, N. Y.

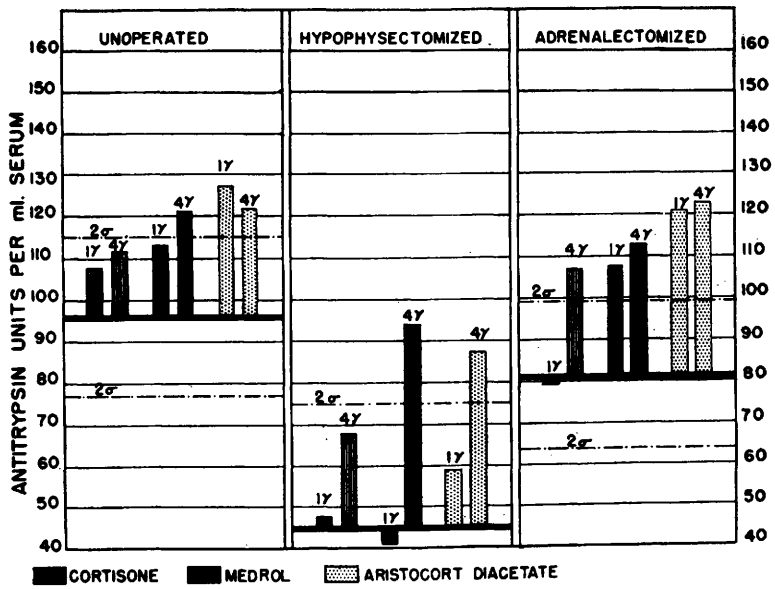


FIG. 2. Alterations in antitryptic activity 72 hr after steroid administration.

only at the higher dosage. Aristocort diacetate administration resulted in a slight increase in serum antitryptic activity at 1 μg, and in a substantial elevation when 4 μg were administered.

Adrenalectomized rats. At 1 μg/g body weight, cortisone administration produced no change in antitryptic serum activity. When a dosage level of 4 μg/g body weight was employed, a pronounced rise in antitryptic activity was evident. With Medrol administration, the increased antitryptic activity noted at a level of 1 μg was equivalent to cortisone induced changes at 4 μg/g body weight. Increasing the dosage level to 4 μg resulted in only a slight elevation over that observed at the lower level. The most pronounced elevations were noted with Aristocort diacetate administration where both dosage levels produced similar increases in antitryptic activity.

Discussion. As in the previous studies(4, 5), it was noted that adrenalectomy is associated with a lowering of the antitryptic activity (Fig. 2). Hypophysectomy produced the lowest level, which is in agreement with earlier work(4). This pronounced decrease, definitely below that observed after adrenalectomy, would tend to indicate that control of serum antitryptic activity is not solely an adrenocortical function, but that other endocrine systems are probably also involved. The data indicate that Aristocort diacetate produced the most consistent elevation in serum antitryptic activity. Medrol appeared to be next in order of effectiveness while cortisone-induced changes were apparent mainly at the higher dosage levels. No attempt was made to equate the dosage of these compounds on a molecular weight basis. It has been shown that Medrol(7) and Aristocort diacetate(8) are several times more active than cortisone with regard to glycogen deposition and anti-inflammatory activity. The great effectiveness of Medrol and Aristocort diacetate in promoting increased serum antitryptic activity suggests that these steroids may be useful in management of clinical hemorrhagic conditions associated with fibrinolysis.

Summary. The influence of cortisone, Medrol (Δ^1-6α-methylcortisol), and Aristocort diacetate (16α-hydroxy-9α-fluorocortisol-16α, 21 diacetate) administration on serum antitryptic activity, at either 1 or 4 μg/g body weight, was studied in normal, hypophysectomized and adrenalectomized rats. The most consistent elevations of antitryptic activity were demonstrated with Aristocort diacetate administration. Medrol proved to be quite effective, while cortisone induced changes were apparent only at the higher dosage levels.

The low serum antitryptic activity observed after hypophysectomy when compared with adrenalectomy would indicate that in addition to the pituitary-adrenal cortex axis other endocrine systems may be involved in the physiological control of the fibrinolytic system.

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Increased Avidity of Antibody for Baltimore 1957 Strains of Asian Influenza Virus (P-Q-R Variation)* (24043)

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Influenza A viruses isolated in Asia during early stages of the 1957 pandemic appeared to have poor antigenic potency. Serum antibody titers of patients convalescent from Asian influenza were lower than anticipated (1), and considerable doubt was cast on the efficacy of the new vaccines because they seemingly failed to induce significant antibody responses. Alternatively, these findings could be explained by insensitivity of viral antigens used to measure antibody titers. It had previously been shown that antigenically related influenza A' viruses often differed markedly in their capacity to combine with specific antibody, a phenomenon known as the P-Q-R variation(3,4). That Asian influenza viruses might undergo the same transformation was implied by Meyer, *et al.*(1) who noted that "certain of the early passage egg fluids of strain A-Japan-305-57 were not readily inhibited by homologous immune serum and appeared to be in Q phase." This supposition is confirmed by serologic studies herein reported, which demonstrate that influenza viruses isolated from patients in Baltimore during autumn of 1957 were in the R phase, although indistinguishable antigenically from the prototype Asian influenza strain A-Japan-305-57.

Materials and methods. Viruses. The prototype Asian strain of influenza A virus, A-Japan-305-57, was obtained from Walter Reed Army Medical Research Institute as lyophilized allantoic fluid of 5th consecutive egg passage. Eight additional strains of virus used were isolated in Oct. 1957 from patients at Johns Hopkins Hospital and are designated by convention as A-Baltimore-1001-57 to A-Baltimore-1008-57. Six of these viruses were recovered from throat washings of patients with clinical manifestations of acute influenza and the remaining 2 from lung tissue of patients who died several days after onset of illness. Penicillin and streptomycin were added to the throat washings or 10% suspensions of lung tissue and 0.1 ml of each material was inoculated into amniotic cavity of chicken embryos 8 days of age. After incubation for an additional 5 days at 37°C the embryos were chilled, amniotic fluids harvested and the presence of virus determined by capacity of fluids to agglutinate an equal volume of 1% guinea pig erythrocytes. Each virus isolate was passaged an additional 1-3 times by the allantoic route in 10 or 11-day-old chicken

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