Metabolism of Corticotropin in Man.* (30148)

ROBERT L. WOLF, MILTON MENDLOWITZ, LOUIS J. SOFFER, JULIA ROBOZ AND STANLEY E. GITLOW
Department of Medicine and the Andre Meyer Department of Physics, Mount Sinai Hospital, New York City

Few data have been published concerning the metabolic fate of intravenously administered corticotropin in man and in animals(1-8). Sayers *et al* found that porcine corticotropin disappeared rapidly from the plasma of man and reached control levels 2 hours after the porcine corticotropin infusion was terminated(1). The plasma disappearance half-times of the porcine corticotropin in their experiments were calculated to range between approximately 20 and 90 minutes. Greenspan *et al* reported a plasma disappearance half-time value of purified adrenocorticotropic hormone in the rat of 5½ minutes(2). Employing I^{131} labeled ACTH (Armour), Sonenberg *et al* reported a blood disappearance half-time value of 4 minutes in the rat (3). Utilizing rat adrenocorticotropic hormone, Richards *et al* described the blood disappearance half-time in the rat to be approximately 3 minutes(4). The blood disappearance half-time of porcine ACTH was found to range between 4 and 18 minutes in normal subjects by Meakin *et al*(5). Cats *et al* described the blood disappearance half-time of corticotropin labeled with I^{131} (Organon) in the rat to be between 5 and 10 minutes (6-8) and Yalow *et al* found the "half-life" of endogenous human plasma ACTH to be less than 10 to 15 minutes(9).

Considering (a) the relatively wide range of values for homologous and heterologous corticotropin plasma (blood) disappearance half-times in man and in animals and (b) the availability of synthetic $beta_{1-24}$ corticotropin,† it was deemed desirable to investigate the plasma disappearance half-time of this tretracosapeptide labeled with I^{131} in man. This report presents results of these studies.

Methods. $Beta_{1-24}$ corticotropin was iodinated by the Newerly modification of the Pressman and Eisen method(10). The $beta_{1-24}$ corticotropin-I^{131} was passed through a Seitz filter and cultured before use in order to insure sterility. Preparations containing less than 0.1 iodine atom per molecule of $beta_{1-24}$ corticotropin-I^{131} were used. The specific activity of the $beta_{1-24}$ corticotropin-I^{131} preparations at time of use was 100 to 150 mc/mg. A dose of 50 to 150 μc of $beta_{1-24}$ corticotropin-I^{131} was injected intravenously in normal subjects. Cumulative urine collections were obtained thereafter. Heparinized blood samples were drawn without tourniquet stasis from the patients at frequent intervals after intravenous administration of the $beta_{1-24}$ corticotropin-I^{131}. Radioactivity assays were performed on plasma and urine in a 5 ml-capacity well-type, scintillation counter with a sensitivity of 0.92 $\times$ 10^6 counts per minute per μc I^{131} above a background of approximately 175 counts per minute. Blood concentrations of radioactivity were plotted as the product of the fraction of the administered dose per liter of plasma and the body weight in kilograms.

Aliquots of the plasma and urine were also analyzed by both paper radioelectrophoresis and paper radiochromatoelectrophoresis and compared with standard solutions of $beta_{1-24}$ corticotropin-I^{131} and I^{131}. Paper radioelectrophoresis was performed in barbital buffer, ionic strength 0.1, pH 8.6. Ten to 100 μl of material were applied to the cathode ends of strips of Whatman No. 3 MM filter paper measuring 37 $\times$ 500 mm which was then stretched across the vertical supports of the buffer vessels. Radioelectrophoresis was effected at 250 volts for 16 to 24 hours after which the strips were dried

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for 50 minutes in an oven at 110°C. Paper radiochromatoelectrophoresis was performed in a similar fashion to paper radioelectrophoresis except that the top of the apparatus was open and the voltage supply was run at 600 volts. By this technique hydrodynamic forces produced a movement of the buffer fluid toward the center of the paper strip from the buffer vessels and the material which was to be analyzed migrated toward the anode from the area of application at the cathode end of the paper strip. Naphthalene black stain and 10% acetic acid in methyl alcohol were employed to color the proteins on the paper strips.

The strips were assayed for radioactivity in an automatic strip scanner employing a thin window gas flow counter with a sensitivity of 0.6 $\times$ 10^6 counts per minute per μc I^{131} above a background of 16 counts per minute. The detector was connected to a linear ratemeter with an integrating count circuit and a dual channel recorder. By these techniques several peaks of radioactivity were usually detected on the plasma radiochromatoelectrophoretograms and the areas beneath the peaks were automatically integrated. The amount of $beta_{1-24}$ corticotropin-I^{131}, therefore, was easily calculated.

Results. When 20 to 50 μl aliquots of $beta_{1-24}$ corticotropin-I^{131} were analyzed by paper radiochromatoelectrophoresis immediately after addition of 20 μl normal human serum or plasma several separate peaks of radioactivity were always detected (Fig. 1A, B). When the paper was not presoaked with $beta_{1-24}$ corticotropin (Fig. 1A) a symmetrical peak of radioactivity was always present at the origin. A second peak of radioactivity (asymmetrical) always migrated with the radiochromatoelectrophoretic mobility of the serum proteins. A third symmetrical peak of radioactivity migrated with the radiochromatoelectrophoretic mobility of I^{131}.

When the paper was presoaked in a solution of $beta_{1-24}$ corticotropin prior to the radiochromatoelectrophoretic procedure in order to saturate the binding sites, the peak of radioactivity that was present at the origin when the paper was not presoaked with $beta_{1-24}$ corticotropin (Fig. 1A) migrated with the radiochromatoelectrophoretic mobility of the proteins (Fig. 1B).

When the $beta_{1-24}$ corticotropin-I^{131} was analyzed by paper radioelectrophoresis several distinct peaks of radioactivity were observed (Fig. 2A, B). When the paper was not presoaked with $beta_{1-24}$ corticotropin, a symmetrical peak of radioactivity was present at the origin and a second asymmetrical peak of radioactivity which migrated with the radioelectrophoretic mobility of the inter beta-gamma globulins was also present (Fig. 2A). When the paper was presoaked with $beta_{1-24}$ corticotropin, the peak of radioactivity which was present at the origin when the paper was not presoaked with $beta_{1-24}$ corticotropin (Fig. 2A), migrated with the radioelectrophoretic mobility of the inter beta-gamma globulins (Fig. 2B).

The peak of radioactivity which was present at the origin when the paper was not

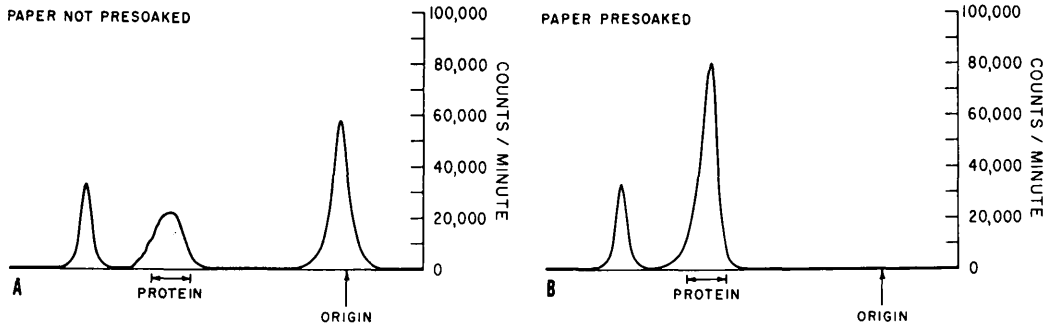

FIG. 1. Paper radiochromatoelectrophoretogram of $beta_{1-24}$ corticotropin-I^{131} in normal human serum. A. Paper is not presoaked with $beta_{1-24}$ corticotropin. $Beta_{1-24}$ corticotropin-I^{131} radioactivity is present at origin. B. Paper is presoaked with $beta_{1-24}$ corticotropin. $Beta_{1-24}$ corticotropin-I^{131} radioactivity migrates with mobility of serum proteins.

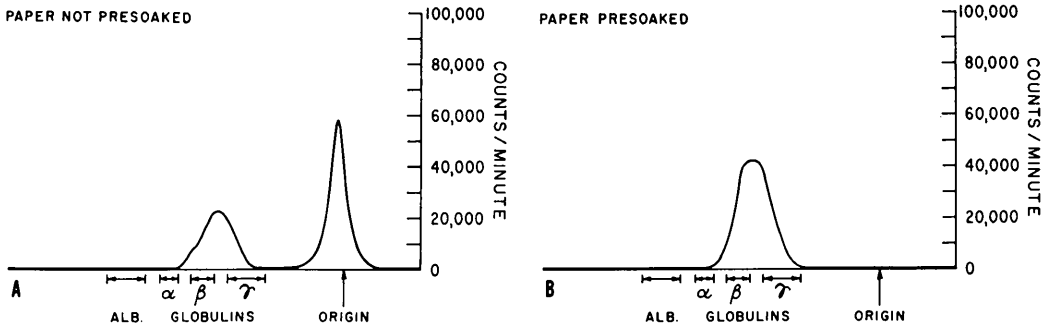

FIG. 2. Paper radioelectrophoretogram of $beta_{1-24}$ corticotropin-I^{131} in normal human serum. A. Paper is not presoaked with $beta_{1-24}$ corticotropin. $Beta_{1-24}$ corticotropin-I^{131} radioactivity is present at origin. B. Paper is presoaked with $beta_{1-24}$ corticotropin. $Beta_{1-24}$ corticotropin-I^{131} radioactivity migrates with mobility of inter beta-gamma globulins.

presoaked with $beta_{1-24}$ corticotropin represented $beta_{1-24}$ corticotropin-I^{131} since the mobility (radioelectrophoretic and radio chromatoelectrophoretic) of this radioactive peak was altered by saturating the binding sites on the paper by presoaking the paper with $beta_{1-24}$ corticotropin and since the mobility of this radioactive peak, after the paper has been presoaked with $beta_{1-24}$ corticotropin, was identical with the electrophoretic mobility of corticotropin(11,12). Furthermore, when this peak of radioactivity was present

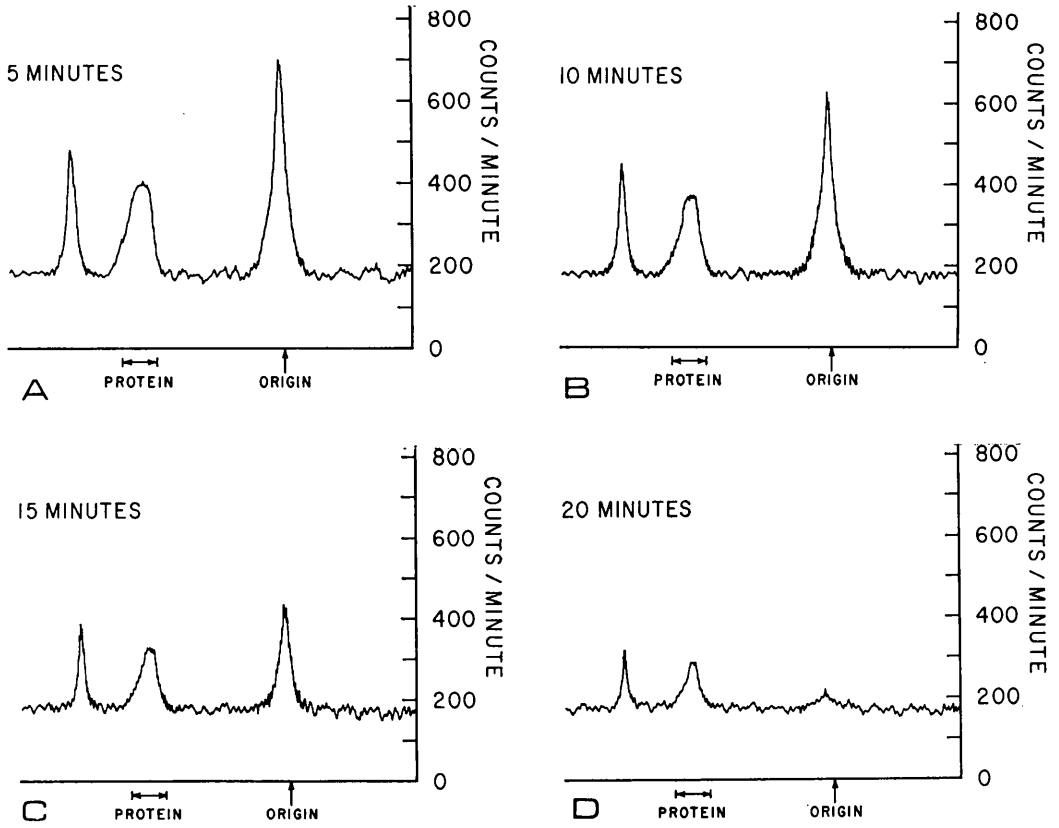

FIG. 3 A, B, C, D. Representative paper radiochromatoelectrophoretograms of plasma samples drawn 5, 10, 15 and 20 min after intravenous administration of $beta_{1-24}$ corticotropin-I^{131} to a normal human subject. $Beta_{1-24}$ corticotropin-I^{131} radioactivity is present at origin.

at the origin (paper not presoaked with beta$_{1-24}$ corticotropin) the radioactive peak was symmetrical without evidence of skewing or distortion.

The peak of radioactivity which migrated the greatest distance from the origin on the paper radiochromatoelectrophoretic strips had the same mobility as I^{131}. When the paper was not presoaked with beta$_{1-24}$ corticotropin, the peak of radioactivity which migrated with the serum proteins when the paper radiochromatoelectrophoretic procedure was employed and which migrated with the inter beta-gamma globulins when the paper radioelectrophoretic technique was used probably represented altered beta$_{1-24}$ corticotropin-I^{131}.

Plasma samples drawn after rapid intravenous administration of beta$_{1-24}$ corticotropin-I^{131} to normal subjects demonstrated that the beta$_{1-24}$ corticotropin-I^{131} radioactivity present at the origin of the paper radiochromatoelectrophoretograms disappeared very rapidly compared to the other peaks of radioactivity (Fig. 3A, B, C, D). The portion of the total plasma radioactivity that was beta$_{1-24}$ corticotropin-I^{131} radioactivity was easily calculated from the total plasma radioactivity and the beta$_{1-24}$ corticotropin radioactivity peak on the paper radiochromatoelectrophoretograms.

Fig. 4 summarizes the beta$_{1-24}$ corticotropin-I^{131} radioactivity in the plasma at various intervals following rapid intravenous administration of beta$_{1-24}$ corticotropin-I^{131} to 9 control subjects. The rapid disappearance rate (plasma half-life) of approximately 7 minutes is apparent. Table I summarizes the apparent space of distribution and the plasma half-life of the beta$_{1-24}$ corticotropin-I^{131} in these experiments.

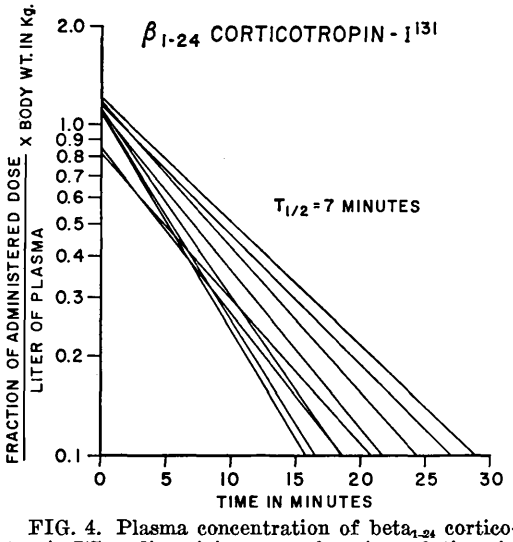

FIG. 4. Plasma concentration of beta$_{1-24}$ corticotropin-I^{131} radioactivity as a function of time in control human subjects.

TABLE I. Plasma Half-Life and Apparent Space of Distribution of Beta$_{1-24}$ Corticotropin-I^{131} in Control Human Subjects.

Subject	Space of distribution % body wt	Plasma half-life T ½ (min)
T.O.	41	6
J.M.	40	7
P.W.	42	5
M.R.	38	10
A.O.	56	7
A.P.	53	6
J.D.	39	9
R.J.	38	8
C.S.	40	5
Mean	43	7

The urinary radioactivity always migrated with the same mobility as I^{131} and probably represented the radioactive end product of the metabolism of beta$_{1-24}$ corticotropin-I^{131}. Ninety-five to 100% of the injected radioactivity was present in the urine in 24 hours.

Discussion. The plasma half-life of synthetic beta$_{1-24}$ corticotropin in control subjects was found to have a mean value of 7 minutes. These results agree with the findings of Meakin *et al*(5) and Cats *et al*(6-8) in both human subjects and rats employing heterologous non-synthetic corticotropin. The mean value for the plasma half-life of synthetic beta$_{1-24}$ corticotropin (7 minutes) in our experiments is also in close agreement with the results of Greenspan *et al*(2) in the rat employing heterologous purified adrenocorticotropic hormone and of Yalow *et al* for human endogenous ACTH(9). The apparent space of distribution of beta$_{1-24}$ corticotropin is approximately equal to that of insulin(13).

The relatively rapid rate of disappearance of beta$_{1-24}$ corticotropin, in comparison with the slow rate of disappearance of the serum proteins, has also been described for other hormones such as aldosterone(14), corticos-

terone(15), hydrocortisone(16) and insulin (13). The rapid rate of disappearance of these hormones and corticotropin from the blood undoubtedly permits a relatively high concentration of these substances in the tissues on physiological demand without undue prolongation of this high concentration when the demand ceases.

Summary. Synthetic beta$_{1-24}$ corticotropin has been labeled with I^{131} and intravenously administered to normal subjects. Analysis of the blood radioactivity following intravenous administration of beta$_{1-24}$ corticotropin-I^{131} revealed that this substance has an apparent space of distribution of 43% of the body weight and a plasma half-life of 7 minutes.

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Suppression of the Homograft Response by Purinethiol Nucleosides.* (30149)

A. P. KIMBALL, G. A. LEPAGE, B. BOWMAN AND S. J. HERRIOT
(Introduced by J. Scholler)
Stanford Research Institute, Life Sciences Research, Menlo Park, Calif.

Reports(1,2) on long term survivals of kidney homotransplants in experimental animals illustrate the feasibility of the chemical induction of immunological tolerance(3) at the organ level. Studies on 6-mercaptopurine and 6-thioguanine(4-9) have demonstrated the effectiveness of these purinethiols as suppressors of the immune reaction. 6-Mercaptopurine acts by blocking the humoral antibody response(9) and may, therefore, act non-specifically. In addition, 6-mercaptopurine and 6-thioguanine produced toxic lesions in the gut and bone marrow(10) which may limit their usefulness as chemical suppressors of whole organ rejections. In general, agents used to inhibit the immune response also suppress lymphoid and myeloid blood elements. The vascularization and health of the graft itself may be drastically inhibited by the generally toxic agents. In this study, it was found that 2 purinethiol derivatives, α-2'-

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