Binding of Hydrocortisone-4-C^{14} and Progesterone-4-C^{14} to Serum Albumin, Demonstrated by Paper Electrophoresis

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Binding of steroid hormones to serum albumin has been studied in several laboratories (*1*). It was found that the maximal binding capacities of albumin solutions for various steroid hormones are in the order of 100 to 10,000 times their physiological concentrations, depending on the nature of the particular steroid concerned. It is evident, therefore, that the potential binding of serum albumin for steroid hormones exceeds by far any concentrations that may occur in the circulating blood, even under extreme conditions. The studies cited, however, do not elucidate to what extent the small quantities of steroids actually present in the blood are bound and transported by albumin, that is, to what degree the binding capacity is utilized. This paper describes experiments on systems in which serum albumin moved, under the influence of an electric field, in the presence of small concentrations of steroids.

The albumin binding was investigated with hydrocortisone, one of the most "polar" compounds known among the natural C_{21} steroids. For comparison, progesterone was selected as a substance that might be considered at the other extreme with respect to the number of polar groups. Hormones tagged with radiocarbon at C_4 were employed (*2*). The activity of the steroids used was such that it permitted determinations with about 10,000 times greater sensitivity than was possible by chemical means. No experimental procedures were applied that might effect structural changes in the steroid molecules; a measurement of the radioactivity, therefore, was equivalent to a determination of the steroid hormones studied.

A purity check of the hydrocortisone-4-C^{14} by paper chromatography showed that the hydrocortisone spot contained 94 percent of the radioactivity. Only 46 percent of the total C^{14} in the progesterone preparation was found in the progesterone peak on a paper chromatogram; rechromatography and isolation from the progesterone spot furnished material of approximately 100 percent purity.

Figure 1 represents the results obtained in a number of experiments. Figure 1 (top) shows that almost the total amount of hydrocortisone-4-C^{14} added to an albumin solution stayed at the origin. The slight cathodic migration was caused by electroosmotic movement of the buffer. Only about 2 percent of the total steroid was transported by albumin and could be demonstrated in statistically significant amounts after extraction of the comparatively large cuts II to V. In contrast, Fig. 1 (bottom) shows that the major part of progesterone-4-C^{14} was bound and transported by albumin under the same conditions of paper electrophoresis. As much as 90 percent of the total progesterone was found to have been shifted from the starting area to the anodic side. This is a minimal value, since no correction was made for the electroosmotic flow in the opposite direction, which was observed in all experiments.

Essentially the same results were obtained with solutions of hydrocortisone and progesterone in human serum and rat serum. The albumin in rat serum transported only about half as much progesterone as did human serum albumin. It may be noteworthy that the albumin in rat serum was found to have only half the azorubin-binding capacity of the albumin in human serum (*3*).

From the location of the C^{14} peaks in the paper-electrophoretic experiments, it seemed possible to conclude that adsorption of the steroids on the paper might interfere with their distribution. Therefore, the adsorptive effect of the paper on the two steroids was studied by paper chromatography in the same buffer that was used in the paper-electrophoretic experiments. It was found that, in the absence of serum

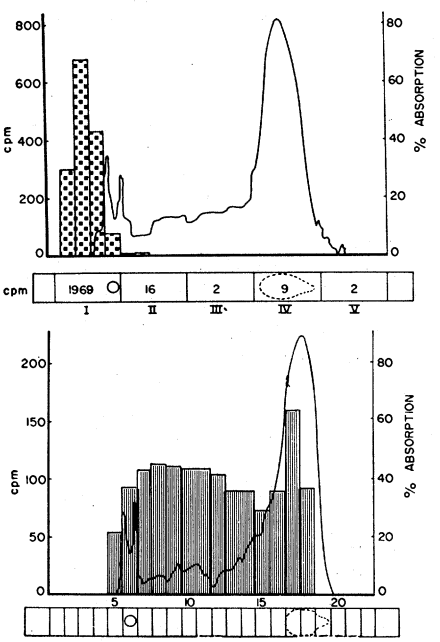

Fig. 1. Paper electrophoresis of solutions of hydrocortisone-4-C^{14} (top) and progesterone-4-C^{14} (bottom) in 4-percent human serum albumin. Concentration, about 50 μg/ml; Michaelis buffer, *p*H 8.6, $\mu = 0.05$. *Abscissas*: Paper strip with cutting lines. The Arabic numerals indicate counts per minute (cpm) for cuttings I to V (top) and the number of cuttings (bottom). The circles on cutting I (top) and on cutting 6 (bottom) represent the spot of application. Cutting IV (top) and cuttings 17 to 19 (bottom) show the albumin. *Ordinates*: Counts per minute in paper cuttings are indicated by bars. Percentage of light absorption of stained albumin is indicated by a solid line. Double peak at spot of application is caused by cover over hole.

albumin, hydrocortisone migrated approximately 3 times as far as progesterone. This observation demonstrated that the considerable transport of progesterone by albumin (Fig. 1, bottom) and the lack of transport of hydrocortisone (Fig. 1, top) could not be the result of different intensities of adsorption on the paper.

The studies show that approximately 2 to 10 percent of hydrocortisone and more than 90 percent of progesterone were transported by and, therefore, were bound to human serum albumin. The hormone concentrations employed were only a fraction of the maximal binding capacities of serum albumin solutions for these compounds. Even at this level, a substantial proportion of the steroids was in free form.

References and Notes

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2. Hydrocortisone-4-C^{14} was generously supplied by the Endocrinology Study Section of the National Institutes of Health; progesterone-4-C^{14} was obtained from a commercial source.
3. U. Westphal *et al.*, *J. Clin. Invest.* **31**, 1064 (1952); ———, *Hoppe-Seyler's Z. physiol. Chem.* **293**, 141 (1953).

10 January 1955.

Continuous Ascending Chromatography—New Technique

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In recent years an impressive list of papers has been published in which the technique of ascending paper chromatography (*1*) has been the primary means of separation or identification. Where the Rf values for the desired components are small, the choice has been continuous descending chromatography or longer sheets of paper for the ascending method with necessarily larger and more elaborate chromatographic chambers. Significant diffusion occurs when the resolving solvent remains in contact with the paper after the solvent front has reached the top of the conventional ascending chromatogram. Lengthening the chromatogram does not adequately circumvent this effect, since the rate of solvent movement is markedly retarded by gravity. The new technique alleviates this shortcoming.

The procedure described here depends on the rapid removal of the solvent as it reaches the top of the chromatogram by capillary ascent (wick effect). Any suitable container is fitted with a glass, cork, wood, polyethylene, aluminum, or stainless steel cap that has one or more slits to permit the top of the ascending paper chromatogram or chromatograms to protrude. Generally, it is sufficient to seal the cap to the jar with Scotch tape. The container, preferably glass, is kept to a minimum height commensurate with the distance that the desired components will travel during the required development. The volume of the container is also kept to a minimum in order to decrease, or essentially eliminate, the time usually allotted for liquid-vapor equilibration (*2*). Ideally, the chamber should approximate the dimensions of the chromatogram. This arrangement may be approached by cutting prescription bottles or other flattened flasks to the desired height. However, spoutless beakers, test tubes, and jars of varying heights are also used. The wick that extends above the cap may be sandwiched between two short wedges of paper in order to seal the opening and to increase the evaporation area.

For most of the solvent systems used in this laboratory, there is sufficient evaporation from the wick under ordinary room conditions to remove the solvent front. For higher boiling solvents systems, the positive draught from a conventional hood or fan is adequate. If evaporation is not sufficient to remove the solvents rapidly, the wick can be bent downward and the solvent front can run off into a beaker. In practice, we have had little occasion to resort to this latter arrangement.

We have found that the new technique is more effective than the continuous descending procedure when there is need for a continuous system. Although our applications have been mainly in the fields of antibiotics and alkaloids, the dye FD&C Blue No. 1 is used simply for illustrative purposes. A commercially available descending chromatographic chamber was equilibrated overnight for the descending technique, and a 250-ml beaker with stainless steel cap was used for the ascending procedure. The FD&C Blue No. 1 was spotted from aqueous solution on Whatman No. 1 paper, air-dried for 5 min, and resolved with methyl cellosolve acetate and water (4 : 1) in the respective chambers. The results are illustrated in Fig. 1.

Typical of other findings is the appearance of three well-defined components in FD&C Blue No. 1 after 18 hr of resolution by the ascending method as against one diffused zone by the descending technique. One

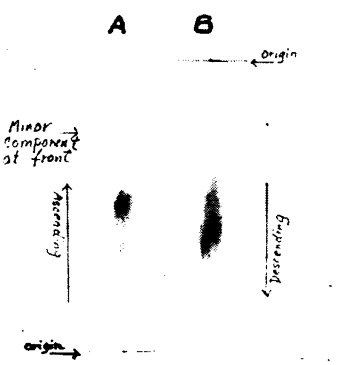

Fig. 1. (*A*) Continuous ascending chromatogram after 18 hr. (*B*) Continuous descending chromatogram after 18 hr.