plate through the hypertrophic zone may also be a factor.

In this experiment, intra-osseous injection of papain caused significant acceleration of bone growth in 26 (41%) of the 63 animals followed for more than one month (Table II). Comparison of these results with the reported effects of various surgical implantations and other operative procedures(11, 13) on bone performed in laboratory animals shows that the highest percentage of animals with significant acceleration of longitudinal bone growth was noted after intra-osseous injection of papain.

Summary. A series of injections of papain protease, in doses of 0.2 or 0.3 mg, was administered into the proximal tibial metaphysis of 89 immature rabbits and the effect on the epiphyseal cartilage plates and on longitudinal growth observed for periods up to 4 months. Twenty-six of 63 animals followed 1 to 4 months had a relative elongation of the injected tibia of 3 to 6 mm. Significant retardation and moderate damage to the epiphyseal cartilage plates of the injected tibia occurred in 3 animals on the higher dosage schedule. Histological studies of the tibial epiphyses a few days after one or two injections of papain disclosed transverse splitting through portions of the hypertrophic zone and a marked increase in thickness of the cartilage plates.

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Interaction of Aldosterone with Liver and Kidney Cell Membranes and Subcellular Fractions of Kidney. (28373)

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The binding of steroid hormones to serum proteins is being actively investigated in an effort to clarify problems of transport as well as of regulation and mechanism of their action. This subject has been reviewed(1,2). The possibility that a correlation exists between the binding of steroid hormones to target tissues and the mechanism of action of such steroids has led to studies of steroid interactions with various tissues and their biological components(3-11).

We have previously reported(12) the investigation of aldosterone binding by serum proteins and the displacing effect of spirolactones and other steroids. It was considered of interest to study the interaction of aldosterone with possible binding sites of its target tissue, the kidney. The binding of aldosterone to kidney and liver cell membranes and to kidney subcellular fractions was determined and compared with the binding of other steroid hormones.

Methods. Male Sprague-Dawley rats weighing 250-300 g and large male New Zealand rabbits were used in these experiments. The animals were sacrificed by a blow on the

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head and were immediately exsanguinated through the carotid-jugular system. The capsules were stripped from the kidneys to allow optimal red cell washout and the kidneys were perfused through the renal vein with saline, pH 7.4. The livers were also perfused with saline, pH 7.4, through the portal vein prior to removal. All experiments were carried out at 0-4°C.

For isolation of cell membranes from the liver and kidney, 50-100 g of tissue were used. The livers were utilized as soon as they had been removed from the animal whereas the kidneys were immediately frozen and processed within 30 days. Comparison of binding results obtained with freshly removed kidneys or with kidneys kept frozen for a month showed no difference. Whole parenchymal tissue was used for isolation of cell membranes obtained from the liver and kidney tissues.

Fig. 1 shows the scheme applied for isolation of liver and kidney cell membranes. The experimental details concerning preparation of homogenates, disintegration of the cells, and procedures for isolating cell membranes are the same as those described by Neville(13). During the preparation the cell membrane fraction was examined for purity by phase contrast microscopy. The final membrane preparation appeared free of contamination or, as in a few preparations, an isolated mitochondrion or nucleus could be seen on a slide prepared for microscopic observation.

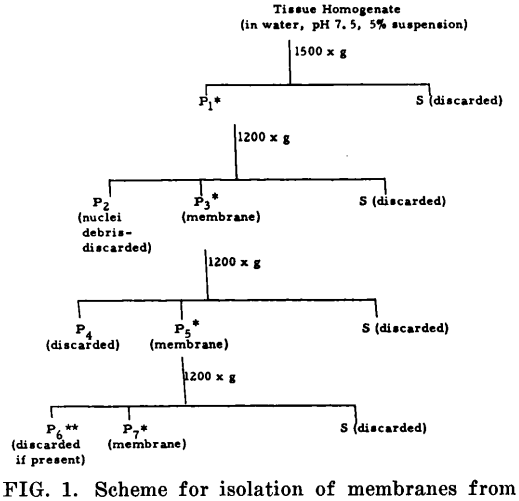

FIG. 1. Scheme for isolation of membranes from liver and kidney cells.

* The precipitate is resuspended in distilled water, pH 7.5, and centrifuged at the stated gravity. For P_3 and P_5, which are layered above P_2 and P_4, respectively, the resuspension is achieved by flotation.

** If P_6 is present or P_7 is contaminated with mitochondria, P_7 is further washed and centrifuged twice more, otherwise the membrane precipitate is resuspended and washed once in phosphate buffer, pH 7.6, μ 0.1.

To study the interaction of aldosterone and other steroids with cell membranes, the method of single equilibrium dialysis was used as described before(12). For the study of the binding of steroids to liver and kidney subcellular fractions (nuclei, mitochondria, microsomes and supernatant) the methods of equilibrium fractionation(11) and multiple equilibrium dialysis were employed (14,15). The cellulose casings containing the cell fractions were dialyzed with gentle shaking at 4°C for a period of 24-48 hours. Previous experiments(11) have shown that these times were sufficient for equilibrium to be attained. The cell membranes suspended in phosphate buffer, pH 7.6, μ 0.1, were dialyzed against this same buffer containing the radioactive steroid. The protein concentration of the membrane preparation was determined by the biuret method and adjusted to 5.0 mg/ml prior to dialysis. After dialysis protein concentration was checked and dry weight determined by drying an aliquot of the membrane suspension in the oven for 48 hours at 100°C, storing in a desiccator for 24 hours and correcting for salt content, calculated from the salt concentration in the evaporated water. The other subcellular fractions were suspended in and dialyzed against 0.44 M sucrose also containing the radioactive steroid. The procedures used for preparation of the dialysis systems, extraction and counting of the radioactive steroids have been described (12).

The following steroids were used: d-aldosterone-7-H^3, specific activity 20 μc per μg; unlabeled d-aldosterone; cortisol-7-H^3, 28 μc per μg; cortisol-4-C^{14}, 42.4 μc per mg; progesterone-16-H^3, 1.8 μc per μg and the spirolactone, SC-9420[3-(3-oxo-7 α-acetylthio-17β-hydroxy-Δ^4-androstene-17 α-yl) propionic

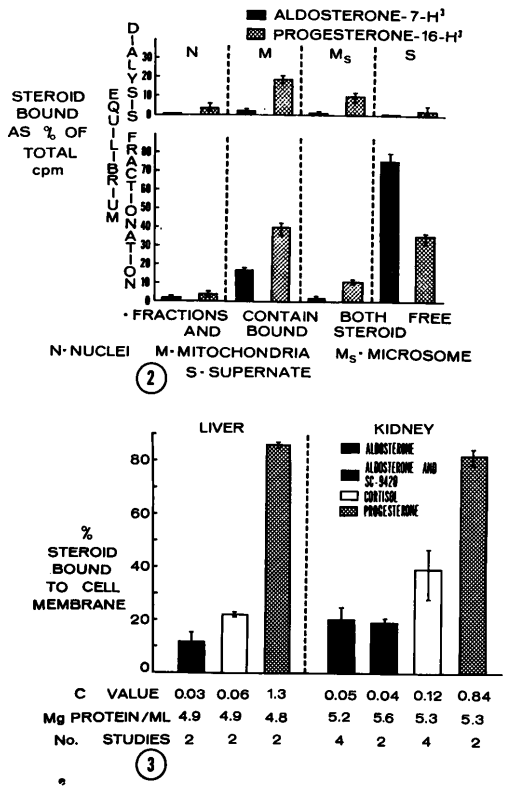

FIG. 2. Interactions of aldosterone and progesterone with rat kidney subcellular fractions. In the equilibrium dialysis, fractions corresponding to 2 g wet kidney (except supernatant which corresponds to 0.4 g) were suspended in 4 ml 0.44 M sucrose in individual cellulose casings and dialyzed against a total of 38 ml 0.44 M sucrose containing 6.0 μg of aldosterone or progesterone. The results are averages of 2 experiments for aldosterone and 4 for progesterone. In the equilibrium fractionation, 6.0 μg of aldosterone or progesterone were equilibrated with kidney homogenate corresponding to a 10% suspension of 5 g wet kidney in 0.44 M sucrose. Fractions were separated by differential centrifugation in equilibrium with the original sucrose solution(11). The results obtained with progesterone are averages of 3 experiments. For aldosterone 6.0, 0.9 and 0.01 μg were used; in the last experiment kidneys from adrenalectomized animals were utilized. In these 3 experiments no significant differences were observed in the percent binding of aldosterone and average values are reported. Range of values is indicated at the top of each bar. For quantities above 0.01 μg for aldosterone and 0.6 μg for progesterone, carrier steroids were added.

FIG. 3. Interaction of aldosterone, cortisol and progesterone with cell membranes from rat liver and rabbit kidney. The bars represent the steroid bound expressed as percent of total activity in the dialysis bag. The combining affinity (C-value) between steroid and protein(22) is calculated as $C = \frac{\text{Bound steroid (cpm/ml)}}{\text{Unbound steroid (cpm/ml)} \times \text{protein conc. (g/l)}}$.

acid γ-lactone]. The steroid concentrations used in these experiments are given in Fig. 2 and 3.

Results. The binding of aldosterone and progesterone to the subcellular fractions of the rat kidney, expressed as percent of total radioactivity (cpm) added to the equilibrium dialysis and fractionation systems, is shown in Fig. 2. No significant binding of aldosterone occurs with the nuclear, microsomal or supernatant fractions, whereas some interaction is demonstrated with the mitochondria. The binding of progesterone by these fractions is greater than that of aldosterone. The binding values for the supernatant fluid in the equilibrium dialysis experiments indicate that the binding of aldosterone or progesterone to the soluble proteins is not significant. It is important to note therefore that the values for the supernatant in the equilibrium fractionation experiment do not represent bound steroid but mainly free steroid.

Further to investigate the binding of aldosterone to cell fractions, the cell membranes of liver and kidney tissues were isolated. Fig. 3 shows the % binding and the combining affinity (C-values) as determined by equilibrium dialysis at 4°C. It is evident that aldosterone interacts with liver or kidney cell membranes with less affinity than either cortisol or progesterone. These results also show that progesterone is more strongly bound than cortisol. In the competition experiments with spirolactone (SC-9420) no displacement of aldosterone from the kidney cell membranes was observed, although the concentration of spirolactone was the same as that which displaced aldosterone from serum proteins(12).

As shown in Fig. 3, the average protein concentration of the cell membrane suspensions is 5.2 mg/ml; where the dry weight of these same suspensions was determined and

The following quantities of steroids were used in the liver experiments: 0.06 μg progesterone-16-H^3, 0.004 μg aldosterone-7-H^3 or 0.75 μg cortisol-4-C^{14} per 30 ml of total buffer solution. In the kidney experiments progesterone and aldosterone were added in same quantities as above, whereas 0.004 μg cortisol-7-H^3 was used. In the competition experiment with kidney cell membrane 0.004 μg aldosterone-7-H^3 together with 340 μg of spirolactone SC-9420 were added per 30 ml buffer. Range of values is indicated at top of each bar.

corrected for salt content, the average value obtained was 7.4 mg/ml. Both liver and kidney cell membranes have a protein content, as determined by the biuret method, of 66% of the dry weight; this agrees with the value of 65% reported for skeletal muscle cell membrane(16).

In other experiments rabbit kidneys were homogenized in phosphate buffer of pH 7.6, μ 0.1, and brought to a protein concentration of 5 mg/ml. This unfractionated homogenate was dialyzed against the same buffer containing aldosterone, cortisol or progesterone of the same concentrations as those used for the cell membrane studies. The steroid bound to the total homogenate expressed as percent of the total radioactivity in the dialysis bag was 24%, 56% and 76% for aldosterone, cortisol and progesterone, respectively, similar to the values obtained with the isolated cell membranes.

Discussion. The mechanism of action of aldosterone on renal and other tissue cells is unknown. The possibility that this action may be facilitated by a local concentration of the steroid in one of the cell fractions which could be isolated was investigated, with particular emphasis upon the membrane of the cell. With all reservations for *in vitro* studies, the results shown in Fig. 2 and 3 appear to indicate that none of the cell fractions investigated "concentrate" aldosterone. The other steroids used for comparison, cortisol and progesterone, were found to be more strongly bound (or easily dissolved) than aldosterone. Since it has been indicated that aldosterone acts predominantly upon the distal nephron(17,18), it is possible that a binding site for aldosterone exists on only a few renal cells or on a particular portion of the membrane or fraction of the cells. The low binding values reported here for aldosterone to renal cell fractions could be due to a low concentration of any specific binding site. However, our data do not seem to substantiate the concept of a particular binding site for aldosterone to kidney cell membranes since similar binding values were observed in the comparative study performed on liver tissue. Fig. 2 shows that the binding of aldosterone to the kidney cell fractions is also very low. These results indicate that if the mechanism of aldosterone action on the distal tubules involves a firm binding of the steroid to the membranes, such attachment cannot be demonstrated by *in vitro* experiments of the present type.

From the results in Fig. 2 and 3 the binding of the steroids studied are in agreement with their solubility properties and follow the polarity rule(2). In comparison to aldosterone, progesterone is more lipophilic and contains less polar groups, qualities which generally result in a stronger affinity to serum proteins and cell constituents(11). Various investigators(16,19,20) have reported that the lipid content of cell membranes and mitochondria is between 15 and 40% of their dry weight. In our studies the kidney and liver cell membranes were found to have a protein content of approximately 66% of their dry weight; a considerable portion of the remainder would undoubtedly consist of phospholipids. It was not determined in the present studies whether the steroids investigated are dissolved in the lipid fraction or bound to the proteins. The theories of steroid action and cell surfaces have been reviewed (21).

In the competition experiments between spirolactone and aldosterone using renal cell membranes, spirolactone (SC-9420) failed to affect aldosterone interaction with the membrane. These results do not support the assumption that the anti-aldosterone effect of the spirolactones is mediated by a displacement of the hormone from a specific attachment to the kidney cell membrane.

Summary. 1. A simplified method for isolation of kidney and liver cell membranes is reported. 2. Aldosterone is weakly bound to kidney subcellular fractions as demonstrated by the techniques of equilibrium fractionation and equilibrium dialysis. 3. The liver and kidney cell membranes studied were found to either take up the steroids according to their lipid solubility or to bind them in accordance with the polarity rule for steroid-protein interaction. 4. Spirolactone failed to displace aldosterone from the kidney cell membranes.

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Passage of Bacteriophage ϕX 174 Across the Placenta in Guinea Pigs.* (28374)

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Previous studies(1-3) have shown that following injection of large amounts of bacteriophage into pregnant animals, trace amounts of phage could be demonstrated in foetal blood. For example, Medearis(4) injected 10^8 PFU of bacteriophage ϕX 174 into pregnant mice and recovered 0.0001% of the amount injected from the foetus; this recovered amount was not influenced by injection of pregnant animals with endotoxin or exposing them to excess CO_2. It is possible that in these experiments the very small amounts of virus found in the foetus represent contamination from maternal blood or fluids which occurred during the experimental intervention.

It was the purpose of this study to determine whether transplacental passage of bacteriophage occurs *in vivo* by excluding the possibility of foetal blood contamination by maternal fluids.

Materials and methods. Animals. Pregnant guinea pigs in each trimester of pregnancy were obtained from breeders.

Phage. The preparation and purification of ϕX has been described(5). The stock used throughout these experiments contained 6×10^{11} plaque-forming particles/ml. One

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