explanation for the present observations. Such an event would make it possible for additional urea reabsorption to occur in the distal tubules and collecting ducts and thus provide a satisfactory explanation for the finding of a urea clearance ratio in the late proximal tubule which approximates that for the entire nephron. Since the surface tubules of the dog kidney extend deeply into the renal medulla(9), the diffusion of urea into loop of Henle fluid would be facilitated by the progressive rise of urea concentration which is known to occur from the outer medulla to the tip of the papilla(10). Unfortunately, because the distal tubules are not accessible to renal micropuncture in the dog, it is not yet possible to quantitate urea addition to the loop of Henle fluid, or urea reabsorption by the distal tubule and collecting duct.

In summary, these renal micropuncture data suggest that urea addition to the loop of Henle fluid occurs in the dog as well as in the rat.

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Renal and Hepatic Effects on Potency of Testosterone Propionate in Chicken. (30579)

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The role of the liver in the inactivation of steroids is unquestionable. For instance, in man it is concerned with the inactivation of steroids(1). Estrogen in the rat is inactivated in the liver(2,3). In the chicken, testosterone is partially removed from the circulation by the liver and comb growth provides a sensitive indication of its hepatic inactivation(4). The renal portal system in the chicken kidney drains the blood from the hind limb and allows its direct access to the renal secretory system(5) offering a convenient way to study the role of the kidney in the processing of drugs when injected in the hind limb muscle. Similarly, the role of the liver can be conveniently determined by injecting the material into the gizzard muscle from which blood directly drains into the hepatic portal system. Injections into the pectoral muscle are drained to the general circulation. This unique circulatory system in the chicken provides for a comparison of potencies of active materials injected through these 3 routes, and may yield quantitative estimates of the effects of the liver and the kidney on the injected material.

Estrogenic stilbene derivatives in chickens have different potencies following peripheral, hepatic, and renal routes of administration (6). The subject of the present investigation was whether the testosterone has the same potency variability in chickens following all the above 3 mentioned routes.

Materials and method. Single comb white Leghorn cockerels, 21 days old, were maintained in wire floored cages. They were allowed Purina mash and water *ad lib.* Cockerels were arranged in groups of 10

TABLE I. Effect of Testosterone Propionate on Comb Growth Response Following Different Routes.

	Control	Gizzard	Hind limb	Pectoral
Avg body wt (g)	1705	1617	1750	1713
Avg comb factor units	567	917	1093	1175
" " " " per g of body wt	.333	.567	.625	.635
No. of animals	15	10	10	10

T value is significant at 1% between pectoral and gizzard and between hind limb and gizzard.

each, for pectoral, hind limb, and gizzard injections. A group of 15 cockerels was used for control, 5 for each route. Testosterone propionate was suspended in sesame oil and the concentration was so maintained that .05 cc contained 500 μg of testosterone. Every bird in the group received 500 μg of testosterone by the route specified for the group. Birds in the control group received .05 cc of sesame oil. Injections were given daily for 40 days. Changes in comb size were expressed in the comb factor units (C.F.U. = length in mm $\times$ height in mm divided by 2) after the method of Bernstorf(7). At the end of the experiment birds were weighed and combs were dissected out under ether anesthesia. Comb growth in terms of C.F.U. and weight were determined. For each group average weight of combs with standard error of mean was determined. To adjust the differences in the body weight, the average total number of C.F.U. increase of each group was divided by the average body weight of that group, thus obtaining the average number of C.F.U. increase per gram body weight for each group. We have used 500 μg of testosterone propionate which has been found to cause rapid comb growth(7).

Result and discussion. Birds receiving sesame oil in the control group either by pectoral, hind limb or gizzard route, did not show any difference in rate of comb growth. This enabled us to have just one control group. The result, when derived on the basis of weight of comb, checked very precisely with the one drawn from the comb-factor-units Table I and II. Thus, weight of comb provides an additional way to find out the extent of testosterone activity. The combs in the birds receiving the testosterone propionate by the pectoral and hind leg routes showed increased growth over the controls, but the two groups were not significantly different. The birds receiving testosterone propionate by the gizzard route showed increased comb growth over the controls but this was significantly less than by the other two routes (Table I). This finding supports the work of Lorenz *et al*(6) that the kidney in the chicken is incapable of inactivating the esterified compounds. A part of testosterone injected into the gizzard muscle undergoes hepatic detoxification which will account for its lesser potency *via* this route.

TABLE II. Average Weight of Combs in (g) After 40 Days, with Standard Error of Mean.

Group	N	Avg wt of comb $\pm$ S.E.
Control	15	2.28 $\pm$ .32
Gizzard	10	3.84 $\pm$ .33
Hind limbs	10	6.20 $\pm$ .71
Pectoral	10	7.54 $\pm$ .75

T value is significant at 1% between pectoral and gizzard and between hind limb and gizzard.

Summary. Our data indicate that the weight of the comb is a good parameter to find out the extent of testosterone propionate activity, as the increase in C.F.U. and the lack of difference between the amount of comb growth when the testosterone propionate is injected by the pectoral or by the hind leg route indicate that there is no detoxification of testosterone propionate in the chicken kidney. The liver plays a major role in its detoxification.

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Intestinal Disaccharidase and Alkaline Phosphatase Activity in the Dog.* (30580)

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Studies on the intestinal disaccharidases of the rat, rabbit, pig and cow have been concerned principally with lactase activity and have demonstrated that the enzyme activity reaches its peak soon after birth and then diminishes in adult life(1-5). There is little information regarding the disaccharidases of the dog(6,7). The present study was undertaken to determine the lactase, sucrase, maltase and alkaline phosphatase activity in the small intestinal mucosa of the developing and adult dog.

Material and methods. Five litter mates (Monseybrook breed) were used for the studies on the young dogs. An animal was sacrificed by exsanguination at 5, 12, 20, 29 and 61 days after birth. The small intestine was rapidly removed from the abdominal cavity and sections were taken one inch distal to the gastroduodenal junction (proximal section), the exact middle (middle section) and one inch proximal to the ileocecal junction (distal section). The sections were opened, rinsed and frozen on dry ice. The intestine from 7 adult mongrel dogs (A1-A7) was removed while the animals were under nembutal anesthesia and similar segments were removed and handled in the same manner. In addition, sections were removed half way between the proximal and middle sections (proximal ¼th) and between the middle and distal sections (distal ¼th) in some of the adult dogs. The tissue was homogenized (25 mg/ml water) and centrifuged in the cold. Disaccharidase activity was assayed by the method of Dahlqvist(8). Aliquots of the supernate were diluted 1:2 for lactase, 1:5 for sucrase and 1:40 for maltase assays. The glucose oxidase reagent used was Glucostat X4 (Worthington Biochemical Corp., Freehold, N. J.). The pH optimum for each disaccharidase was determined by measuring enzyme activity in a wide range of pH's using a 0.05 M sodium acetate buffer from pH 3.6 to 4.5, a 0.056 M maleate buffer from pH 5.0 to 7.0 and a 0.025 M sodium veronal buffer from pH 7.5 to 8.0. The necessary pH adjustments were made using HCl or NaOH and a Beckman pH meter. All analyses were performed in duplicate and the means of these 2 figures were taken for presentation of the results. The disaccharidase activity was expressed in units (micromoles of disaccharide hydrolyzed)/min/g wet weight. Alkaline phosphatase activity was determined by the method of Klein *et al*(9) and expressed as K.R.B. units/30 min/g wet weight (Phosphatabs—Quantitative Alkaline Substrate Tablets, Warner-Chilcott, Morris Plains, N. J.).

Results. Optimum pH. The optimum pH was found to be pH 5.0 for lactase activity and pH 6.0 for sucrase and maltase activity. The 0.056 M maleate buffer and these pHs were used for all of the following experiments.

Changes according to age. Alkaline phosphatase was high in all 3 sections of small intestine from the youngest dogs and decreased to adult levels in the proximal section by 29 days, Table I. The activity of this enzyme in the middle and distal sections obtained from the 29- and 61-day-old pups was still higher than that noted in the same sections from the adult dogs. Lactase activity also was the highest in the intestine of the youngest dogs and decreased to the levels

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