phate buffer solution or a saline solution. The active factor precipitated from the phosphate buffer system along with phosphate salts after addition of ethanol and butanol and adjusting the pH to 12.5. Phosphate salts were subsequently removed by dialysis, during which mammogenic hormone precipitated out of solution. Active factor was precipitated from saline solution by saturation of solution with NaCl and chilling. Resulting light grey, amorphous powder obtained by phosphate extraction was 223 times more potent in promoting mammary gland growth than progesterone, and contained negligible, if any, lactogenic activity. A highly purified lactogenic preparation assayed for mammogenic activity at the same time was only 1.59 times as potent as progesterone. On this basis the lactogen need contain no more than 0.71% of mammogen to produce the biological response observed.

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Effect of Corticotropin and Growth Hormone on Allantoin Synthesis and Excretion in the Dog.* (25878)

H. C. CHOITZ AND O. H. GAEBLER
Edsel B. Ford Inst. for Medical Research, Henry Ford Hospital, Detroit, Mich.

The effect of corticotropin on excretion of end products of purine metabolism seems to vary with species. In dogs urinary output of allantoin is increased by massive doses of corticotropin, presumably as a result of accelerated excretion as well as increased allantoin synthesis(1). On the other hand, corticotropin apparently does not increase allantoin synthesis in the rat, and the renal tubule has been suggested as a possible site of action of hormone on allantoin excretion(2). Likewise uricosuria in human subjects after administration of corticotropin has been attributed to increased renal clearance of uric acid rather than increased synthesis(3). A recent investigation of effects of corticotropin and growth hormone on utilization of N^{15} from individual sources(4) afforded an opportunity to secure data on incorporation of the isotope, ingested as labeled glycine or ammonium citrate, into urinary allantoin of normal dogs, during control experiments, and after administration of corticotropin or growth hormone. Experiments with growth hormone seemed relevant because of its importance in nitrogen metabolism in general, and in view of reports of its positive action on mitotic activity and synthesis of nucleic acids(5,6).

Materials and methods. Two adult mongrel bitches were used as experimental animals. Details regarding care, diet, source of hormones, and preparation of labeled compounds have been described(4). Corticotropin was given subcutaneously in 2 doses of 10 units each (9:00 a.m. and 3:00 p.m.) for 3 successive days. Growth hormone, in doses of 5 mg dissolved in 1 ml of normal saline, was administered subcutaneously on 4 consecutive days. The labeled compound was fed with the morning ration on second day of corticotropin treatment and on third day of growth

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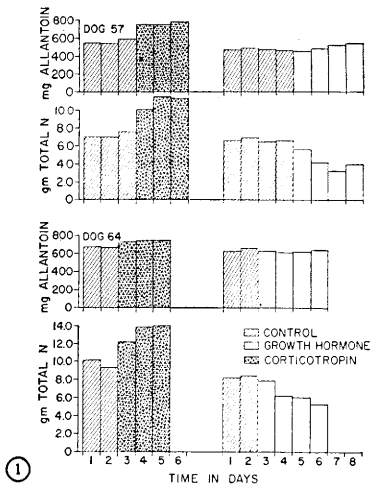

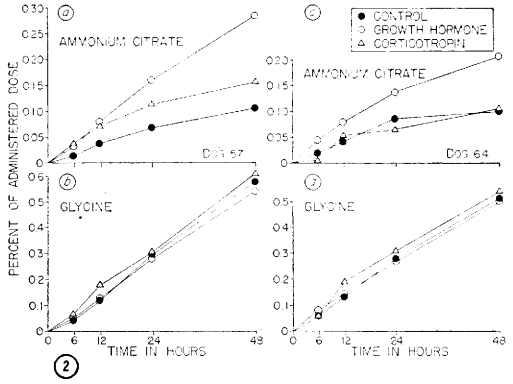

FIG. 1. Effect of corticotropin and growth hormone on excretion of total nitrogen and allantoin.

FIG. 2. Incorporation of N^{15} from ammonium citrate and glycine into urinary allantoin, expressed as cumulative % of administered dose: *a*, *b*, Dog 57; *c*, *d*, Dog 64.

hormone therapy. Urine was collected under toluene at 6, 12, 24 and 48 hour intervals after tracer had been fed. Each collection period was terminated by catheterizing and washing the bladder. Nitrogen of allantoin was isolated as the dixanthylurea derivative in a manner similar to that of Allen and Cerecedo (7). In this method, preexisting urea is first decomposed with urease. Allantoin is then successively hydrolyzed with base to allantoic acid, and finally with acid to urea and glyoxylic acid. Addition of xanthydrol to the acid solution precipitates urea formed. To a sample of urine, which would yield at least 0.5 mg of allantoin nitrogen, were added 5 ml of phosphate buffer, pH 7.0, and 4 ml of urease solution, after which incubation was carried out at 40°C for 2 hours. The sample was then made alkaline to about pH 12 with 50% sodium hydroxide, and heated on steam cone for 20 minutes. After cooling, sample was acidified to about pH 2 with concentrated hydrochloric acid, treated with decolorizing carbon, heated on steam cone for 10 minutes, and filtered hot. The filtrate was treated with enough xanthydrol dissolved in methanol-glacial acetic acid (80:20) to precipitate the urea. The precipitate was collected by centrifugation, washed successively with water, alcohol, and ether, and dried at 110°C. Procedures for digesting the compound, distilling the ammonia obtained, and analyzing its nitrogen in the mass spectrometer were the same as in preceding study(4). In several preliminary trials, acidification of incubation mixture and addition of xanthydrol gave no alcohol-insoluble precipitate, indicating that decomposition of urea originally present as such was complete. Recovery of allantoin nitrogen was not quantitative, but averaged about 60% of that found colorimetrically by the method of Young and Conway(8).

Results. Corticotropin increased urinary excretion of allantoin, while growth hormone had little or no effect (Fig. 1). The increase was much more marked in Dog 57 than in Dog 64. A minor part of this difference may have been due to the fact that Dog 57 weighed about 3 kilos less than Dog 64, while both received the same dosage of corticotropin. In all experiments treatment with corticotropin or growth hormone produced the expected decrease or increase in total urinary nitrogen.

Amounts of N^{15} incorporated into urinary allantoin during 48 hours after feeding labeled compounds, and specific enrichments (μg N^{15}/g allantoin N) are given in Table I. Values for specific enrichment represent average of entire period. The nitrogen of glycine was utilized to considerably greater extent than that of ammonium citrate for synthesis of allantoin, since almost identical amounts of isotope were fed in each case.

Corticotropin did not significantly alter specific enrichment of allantoin nitrogen. A possible interpretation of this result is that in-

TABLE I. Incorporation of N^{15} into Urinary Allantoin in 48 Hours.

Dog	Source* of N^{15}	Treatment	N^{15} incorporated μg	$\mu g/g$ allantoin N
57	Amm. cit.	None	23	62
"		Growth hormone	61	139
"		Corticotropin	34	63
64		None	21	43
"		Growth hormone	44	103
"		Corticotropin	23	45
57	Glycine	None	126	316
"		Growth hormone	117	307
"		Corticotropin	133	289
64		None	111	221
"		Growth hormone	109	223
"		Corticotropin	118	224

* Ammonium citrate-N^{15} contained 21.46 mg N^{15}, and glycine-N^{15} contained 21.65 mg N^{15}.

creased excretion reflected increased synthesis of allantoin, but that the proportion of unlabeled and labeled precursors involved in this process remained the same as in control experiments.

Excretion of N^{15} in allantoin nitrogen, expressed as cumulative per cent of administered dose of N^{15}, is illustrated in Fig. 2. Similar results were obtained with both dogs. There was a relatively large increase in utilization of ammonium citrate-N^{15} for allantoin synthesis when growth hormone was given. Since no comparable increase occurred in the case of glycine-N^{15}, this may be a reflection of hormone's effect, or lack of effect, on pool size of different allantoin precursors.

It has been suggested that there are in the human 2 pathways for incorporation of labeled precursors into urinary uric acid(9,10). One pathway presumably proceeds *via* nucleotide intermediates, which are oxidized to uric acid before they can be incorporated into nucleic acids. In the second pathway, the labeled precursor finds its way into cell nucleic acids. The labeled purines are then oxidized to uric acid as cells break down. Any uric acid arising from first pathway would be labeled more rapidly than that formed from breakdown of nucleic acids, since turnover rate of the latter is slow.

It cannot be definitely concluded from our data that a similar situation exists in the dog. However, prompt appearance of ingested N^{15} in urinary allantoin might suggest nucleotides, rather than nucleic acids, as principal precursors during short interval studied.

Summary. 1. Growth hormone increased amount of ammonium citrate-N^{15} utilized for allantoin synthesis in dogs. Corticotropin did not have this effect. Neither hormone increased incorporation of N^{15} from glycine into allantoin. 2. Corticotropin increased output of urinary allantoin in dogs, without markedly reducing the specific enrichment with N^{15} in this excretory product. One possible interpretation of this finding is increased synthesis of allantoin, without qualitative changes in its origin.

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