that the higher salivary tryptophan level observed in caries-immune humans(4) is not the only factor involved in caries immunity in humans and that a similar immunity to caries cannot be induced in rats by feeding tryptophan.

The daily weight gains in the animals receiving the tryptophan were somewhat less. However, these differences were insignificant in all groups except in the one receiving 5.4 g tryptophan per kg of diet. In this group the final weight of both the males and the females was about 20% less than the controls.

The role of protein degradation in the oral cavity or within the salivary glands themselves must bear some relationship to caries immunity in the rat, since the salivary gland-thyroid studies clearly indicate an increased proteolytic effect of the salivary gland homogenates following administration of desiccated thyroid, while a marked decrease resulted following administration of I^{131} or thiouracil. The latter substances are known to be related to caries immunity or susceptibility, respectively. The anatomical structures within the salivary gland affected by thyroid activity are the serous tubules, and Shafer‡ has noted that following thyroxine administration large numbers of the granules pass into the excretory ducts of the salivary glands. Since these granules are thought to be predominantly protein in nature, the significance of proteolytic enzymes, protein metabolism, and the role of different amino acids on caries resistance or susceptibility in the rat needs much further study.

Summary. Dental caries experience in rats was studied following feeding of varying levels of DL-tryptophan as a constituent of a cariogenic diet. When either 0.6 or 1.8 g/kilo tryptophan was added to the diet there was an increase in dental caries, although the differences were not significant. When the diet contained 5.4 g/kilo tryptophan there was a significant increase in incidence of dental caries. Concomitant with the increased dietary tryptophan level, there was an increased salivary tryptophan concentration.

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Status of Deoxycorticosterone as Intermediary in the Biosynthesis of Cortisol.* (23204)

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The conversion of cholesterol to 17α-hydroxycorticosterone (cortisol) and corticosterone, demonstrated in adrenal preparations (1,1a,2,3) has been postulated to proceed via the reaction sequence shown in Fig. 1(1,4). All of the individual reactions shown have been definitively demonstrated in cell-free systems, and the available *in vivo* data are consistent with this scheme(4). The postulation of an orderly series of hydroxylations of the progesterone molecule was derived in large part

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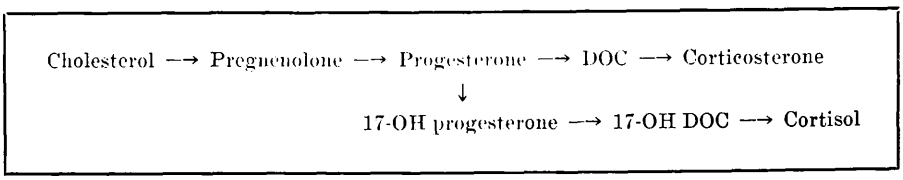

FIG. 1. Corticosteroidogenic sequence.

from the bovine perfusion findings that while deoxycorticosterone (DOC) was converted to corticosterone, neither DOC nor corticosterone could be further hydroxylated at C-17α to form cortisol; subsequent studies, using homogenates from bovine and porcine adrenals, yielded essentially similar results(5,6,7,8). Recently, however, it has been reported by Heard and his associates(2,3) that C^{14}-DOC is readily converted in good yield to cortisol as well as corticosterone in adrenal preparations from a variety of species including bovine. Heard *et al.* have therefore concluded that the sequence of hydroxylations postulated in Fig. 1 is not obligatory and that alternative routes are operative to a major degree. To clarify the apparent discrepancy between the work of Heard's group and the previous findings cited, the role of DOC as an intermediary has been reinvestigated in cow adrenal homogenates which form corticoids from endogenous precursors. For this purpose tracer amounts of DOC-21-C^{14} and progesterone-4-C^{14} were incubated with homogenates prepared in various media, and the degree of incorporation into the cortisol and corticosterone formed was measured. In all homogenates studied, it was observed that while DOC-21-C^{14} was incorporated into the corticosterone, no incorporation into the cortisol formed in the system could be detected; progesterone-4-C^{14}, on the other hand, was incorporated into both cortisol and corticosterone. While DOC-21-C^{14} was not converted to cortisol, it did give rise to certain unknown C^{14}-products, of polarity similar to but not identical with cortisol, which could be separated from cortisol by careful paper chromatographic fractionation.

Methods. Breis of decapsulated adrenocortical tissue from fresh cow glands were homogenized in each of the following media using a loose-fitting Potter-Elvehjem glass apparatus (4 ml medium per g tissue): (a) 0.025*M* sucrose, (b) 0.154*M* KCl and (c) 0.154*M* NaCl. All homogenizing media were buffered with M/150 Sorensen's phosphate buffer and supplemented with niacinamide, sodium fumarate and $MgSO_4$ (all at a concentration of 5 mM/l). After homogenization ATP‡ and DPN§ were added to a final concentration of 1.0 and 0.5 mM/l, respectively. All operations involved in the preparation of these homogenates were carried out near 0°. Homogenate aliquots corresponding to 35 to 100 g tissue were added to reaction vessels containing 298 to 600 μg of progesterone-4-C^{14} (specific activity (S.A.) 1.60×10^6 cts m/mg) or 332 to 700 μg DOC-21-C^{14} (S.A. 1.55×10^6 cts/m/mg) suspended in about 0.3 ml of propylene glycol, and then incubated for 2 hours at 38° with shaking (gas phase—100% O_2). Aliquots of the same homogenate extracted without incubation served to measure the endogenous cortisol and corticosterone initially present. The C^{14}-progesterone sample used in these experiments was radiochemically homogenous in the ligroin-propylene glycol paper chromatography system(9), and had been further chromatographed on silica gel; after prolonged percolation, benzene-ethyl acetate (20:1) eluted C^{14}-progesterone from the column as a sharp symmetrical peak of radioactivity. The C^{14}-DOC sample used, obtained as the 21-monoacetate, was first purified as the acetate using the toluene-hexane(1:1) - propylene glycol paper chromatography system; when homogenous in this system, the acetate was saponified and the free DOC was chromatographed in the toluene-propylene glycol system until it was radiochemically homogeneous in this system.

‡ ATP—Adenosine triphosphate

§ DPN—Diphosphopyridine nucleotide

TABLE I. Products Isolated from Bovine Adrenocortical Homogenates Prepared in Various Media and Incubated with C^{14}-DOC and C^{14}-Progesterone.

	Substrate*	Substrate added (μg)	Tissue wet wt (g)	Medium	Net steroid production (mg/100 g/2 hr) Cortisol	Net steroid production (mg/100 g/2 hr) Corticosterone	C^{14}-Substrate converted (μg/100 g/2 hr) Cortisol	C^{14}-Substrate converted (μg/100 g/2 hr) Corticosterone
	Progesterone-4-C^{14}	322	35	Sucrose	.89	1.31	18	51
				KCl	.82	.68	3	34
A				NaCl	.77	1.18	4	81
	DOC-21-C^{14}	332	"	Sucrose	.76	1.98	<.5	137
				KCl	1.09	1.04	<.5	45
				NaCl	.82	1.02	<.5	72
	Progesterone-4-C^{14}	298	60	Sucrose	2.58	2.60	52	123
		540	100	"	1.52	2.12	27	76
B		600	46	KCl	1.16	2.09	10	182
	DOC-21-C^{14}	700	44	Sucrose	.76	2.50	<.5	415
		700	46	KCl	1.20	1.52	<.5	145

* S.A.—Progesterone, 1.60×10^6; DOC, 1.55×10^6 cts/min./mg.

Finally, the DOC was chromatographed on silica gel; after percolation of large volumes of a benzene-ethyl acetate mixture (10:1) the DOC was eluted by this solvent mixture as a sharp symmetrical peak. Corticosteroids were obtained from the homogenates by exhaustive extraction with acetone and ethyl acetate; the total extracts were combined and distilled *in vacuo*. The resulting aqueous solution was then thoroughly extracted with ethyl acetate. The lipoid extracts thus derived contained 90-95% of the added radioactivity. After partial removal of phospholipids in the crude extract by acetone precipitation in the presence of $MgCl_2$ (in the cold), the extract was further purified by silica gel chromatography. After eluting nonradioactive materials with hexane, benzene and benzene-ethyl acetate mixtures (9:1), about 75% of the radioactive materials and all of the cortisol and corticosterone present were eluted with a benzene-ethyl acetate mixture (1:1) and with ethyl acetate. The additional radioactivity eluted by methanol (about 25%) did not contain cortisol or corticosterone as determined by paper chromatography. The cortisol and corticosterone present in the silica gel eluates were isolated by procedures previously described (1), wherein the chloroform-formamide and toluene-propylene glycol systems of paper chromatography (9) were utilized for cortisol and corticosterone respectively. The cortisol and corticosterone zones located by ultraviolet scanning and characteristic spotting reagents (TPTZ, Bush soda fluorescence and H_2SO_4 fluorescence tests), were then eluted and rechromatographed in the same systems. The rechromatographed free steroid alcohols were then eluted, acetylated and chromatographed on toluene-propylene glycol (18 hr) and toluene-ligroin (1:1)/propylene glycol (8 hr) for cortisol acetate and corticosterone acetate respectively. The corticosteroid acetates were located by the reagents mentioned previously and then rechromatographed in the same systems. At this stage of purification the specific activities of the corticosterone acetate and cortisol acetate were determined by counting an aliquot (infinite thinness) in a flow-gas counter and determining the corticosteroid content with a modified Mader-Buck procedure using blue tetrazolium. During the entire course of purification, all chromatograms were scanned in a calibrated counting chamber, so that it was possible to locate zones of radioactivity. With the exception of the cortisol acetate zones derived from the DOC incubations, all of the above-mentioned zones contained a peak of radioactivity.

Results. The results obtained in these experiments are shown in Table I. In A, the various homogenates were all derived from the same pool of adrenocortical brei, incubated with both C^{14}-progesterone and C^{14}-

TABLE II. Values at Successive Stages of Purification (Sucrose Homogenate).

Substrate	Specific activity (counts/min./μg) "Cortisol"	Cortisol acetate*	Cortisol acetate†
C^{14}-Progesterone	27	33	38
C^{14}-DOC	63	8	<0.8

* After first chromatogram. † 2nd chromatogram.

DOC and worked up simultaneously. In B, the results shown were obtained in individual experiments using different adrenal tissue. The results in both types of experiment are comparable in that: (a) in none of the homogenates was it possible to detect the conversion of C^{14}-DOC to cortisol, despite the fact that endogenous production of cortisol was clearly evident in all cases; (b) the conversion of C^{14}-DOC to corticosterone proceeds in all homogenates and (c) the conversion of C^{14}-progesterone to both cortisol and corticosterone is demonstrated. These results are fully consistent with the scheme postulated in Fig. 1. The marked effect of the ionic media upon the lesser degree of incorporation of C^{14}-progesterone into cortisol relative to corticosterone will be the subject of a subsequent paper.

While the cortisol finally isolated from the C^{14}-DOC incubation products did not contain detectable radioactivity, considerable radioactivity was observed in the cortisol zone prior to acetylation. Table II shows the changes in S.A. of the "cortisol zone" derived from C^{14}-DOC incubations in a typical sample at various stages of purification by paper chromatography; for comparison the results obtained with the "cortisol" derived from a C^{14}-progesterone incubation are also shown. It will be seen that with the material from C^{14}-DOC incubation a considerable amount of radioactivity is initially associated with the cortisol zone, and that with successive purification by paper chromatography the radioactivity disappears. In marked contrast, the radioactivity of the cortisol zone derived from C^{14}-progesterone remains relatively constant during these same fractionation procedures. While the present homogenate studies are not directly comparable to those of Heard *et al.* (2,3), the present findings raise the strong possibility that in the studies of Heard *et al.* C^{14}-DOC was converted to a radioactive product of mobility similar to cortisol, *which is not cortisol.*

In accord with our conclusion, recent work with rat adrenal tissue(11) has likewise failed to detect the conversion of DOC to cortisol, although it was reported that this steroid was converted to Porter-Silber reactive chromogenic material (presumably containing the dihydroxyacetone side chain characteristic of cortisol) and to a ketolic steroid zone of polarity intermediate between cortisone and corticosterone. Similarly, it has been reported that corticosterone is converted to more polar products, the nature of which has not been defined(10). Thus it seems clear that DOC can be converted in appropriate *in vitro* systems to products which are more polar than corticosterone as well as to corticosterone. However, until it has been shown, using unequivocal technics for the demonstration of radiochemical homogeneity, that DOC is actually converted to cortisol, there would appear to be no need to postulate an alternative pathway of cortisol production via DOC.

Summary. Homogenates of adrenocortical tissue which synthesize cortisol and corticosterone from endogenous precursors were prepared in various media and incubated with trace amounts of DOC-21-C^{14} and progesterone-4-C^{14}. While C^{14}-DOC was converted to corticosterone, and C^{14}-progesterone to both corticosterone and cortisol, C^{14}-DOC was not converted to cortisol. DOC, however, was converted to a C^{14}-product possessing a chromatographic mobility similar to cortisol, which was separated from cortisol by careful paper chromatographic fractionation.

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Meso-Inositol Requirement of HeLa and Human Conjunctival Cells.* (23205)

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A number of different human cells have been shown to require meso-inositol when grown *in vitro*(1). This is of considerable interest since it represents the first conclusive demonstration that meso-inositol is an essential nutrient for human cells. The report of Eagle *et al.*(1) was also of interest because no conclusive evidence was obtained that inositol was required by the cell strain derived from a human carcinoma, the HeLa cell.

In similar studies with the human conjunctival cells(2), regular success was obtained only after a greater concentration of inositol was used than the 1 μM suggested by Eagle *et al.*(1). In view of this, the requirement for inositol by the HeLa cell was also studied. These studies form the basis of the present report.

Methods. Human conjunctival cells(2) and HeLa cells(3) were used throughout these experiments. Stock cultures were propagated in 250 ml serum dilution, neutral glass bottles containing 10 ml of Eagle's basal medium(4) plus 10 to 20% human serum. Only stock bottles which showed the presence of a continuous sheet of polyhedral cells with clear cytoplasm and occasional mitoses were used. The actual experiments were conducted using sets of 4 roller tubes, 18 x 150 mm, containing a total volume of 1 ml of Eagle's medium plus 20% dialyzed human serum. Following inoculation, the tubes were slanted at a 2-5° inclination at 36°C for 16 to 20 hours and then were placed in a roller drum also at 36°C. The medium was replaced in all tubes on the third and fifth days. The cells were examined daily under 100x magnification. On the seventh day, the cells were trypsinized and counted with a Levy counting chamber (5). Dialysis of serum was accomplished as follows: 15 ml of serum was dialyzed in cellulose dialysis tubing† against 15 ml of Eagle's basal medium(4) for 24 hours; to maintain the pH at 7.2 to 7.4, the serum was dialyzed in a stoppered Erlenmeyer flask of 125 ml capacity. The dialysate was frozen at −80°C until used. The serum was dialyzed further against 4 changes of 0.85% NaCl, of about 1 liter each, for a total of 48 hours, and then against approximately 100 ml of Eagle's basal medium for one to 2 hours longer. Each dialysis procedure was carried out at 0 to 2°C with constant agitation. The volume of the dialyzed serum was routinely measured and usually showed an increase of 0.5 to 1.0 ml. The dialysate was routinely checked for serum protein contamination by the addition of

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