of course, not unique. It seems incorrect, in the light of this study, to consider CxRP to be made only when an animal is "sick".

In the postlabelled rabbit (#210), the general similarity between the specific activities of serum albumin and CxRP are consistent with some *de novo* synthesis of CxRP. The specific activity of the CxRP rose initially as newly synthesized labelled protein was added to the animal's total circulating CxRP. After the first few hours, further synthesis of unlabelled CxRP resulted in a reduction in specific activity, while after the tenth hour both the albumin and CxRP declined in specific activity at about the same rate.

Summary. Studies on the kinetics of formation of CxRP in rabbits have been carried out. By measuring relative specific activities of CxRP and serum albumin in rabbits given glycine-1-C^{14} and induced with typhoid vaccine to form CxRP, evidence has been obtained to suggest that CxRP is being manufactured constantly and appears in the serum on challenge.

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Utilization of Free and Esterified Cholesterol-4-C^{14} for Corticoid Biosynthesis by Hog Adrenal Homogenates.* (27582)

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It has been shown(1) that hog adrenal homogenates produce corticoids in the presence of reduced triphosphopyridine nucleotide (TPNH) and oxygen. During steroidogenesis, the level of endogenous free cholesterol declined by an amount comparable to the amount of steroid produced. Adrenal free cholesterol fell to about 50% of the original level during a 2-hour incubation period. However, addition of exogenous free cholesterol to the homogenates initially or after 2 hours, failed to increase steroid production. The level of cholesterol esters in the homogenates remained constant throughout the incubation period. These findings are in agreement with those of Péron and Koritz(2), who, using rat adrenal homogenates, suggested that the failure of exogenous free cholesterol to increase corticoid output could be explained by enzyme saturation by endogenous cholesterol or by an inability of exogenous cholesterol to enter the biosynthetic pathway for corticoid synthesis. Later Péron(3), concluded that mixing of endogenous and exogenous free cholesterol did take place in rat adrenal homogenates. Addition of 117 μg of cholesterol-4-C^{14} to an incubation mixture containing about 35 mg of rat adrenal tissue with a TPNH generating system gave 1.88% of the total C^{14}-radioactivity in eluates of steroid zones after chromatography.

The present work was undertaken to study further the interconversions of free and esteri-

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fied cholesterol and their role as precursors for steroid production in hog adrenal homogenates.

Materials and methods. Homogenates of hog adrenal glands (100 mg ml) were prepared as described previously(1). Cholesterol-4-C^{14} (Nuclear-Chicago Corp.) or cholesterol-4-C^{14} ester dissolved in propylene glycol was added to the homogenates in amounts up to 5 μg per 100 mg of adrenal tissue. This upper level was equal to 1.6% of the free cholesterol present in the homogenates and was considered to be a tracer dose, *i.e.*, it would not change equilibrium conditions in the homogenate. All incubations were carried out in triplicate with 2 series of flasks under identical conditions. One series was used for cholesterol assays and the other for steroids. The mixtures were incubated at 37°C for 2 hours under 95% O_2-5% CO_2. Incubation mixtures for the steroid series contained 1 ml of adrenal homogenate and were made to a final volume of 3 ml with Krebs-Ringer-Bicarbonate buffer, pH 7.4(4). One mg of TPN and 3.0 mg of glucose-6-phosphate (G6P) were added to the experimental flasks to promote steroid production. Incubation mixtures for the cholesterol series contained double quantities and volumes of the above components.

The contents of the flasks of the cholesterol series were extracted with hot acetone-alcohol (1:1) and aliquots of this extract used to determine C^{14}-activity of the sterol digitonides as well as the free and ester cholesterol content of the homogenates(5). C^{14}-activity was determined in the washed sterol digitonides(6) which were dissolved in chloroform-methanol (2:1) and counted at infinite thinness in a gas-flow counter.

The contents of the flasks of the steroid series were extracted with methylene chloride and washed with 0.1 N NaOH and 0.1 N HCl. The extracts were chromatographed on paper in the ligroin-propylene glycol system (7) for 2½ hours. Ultraviolet absorbing zones and those containing C^{14}-activity were located on the strips after drying. These were found to coincide and extended from the origin to about 5 cm below the origin. Adjacent lanes containing standard steroid substances showed that any hydrocortisone, corticosterone, 11-desoxyhydrocortisone and desoxycorticosterone, the major BT reacting compounds produced by hog adrenal slices *in vitro*(8), would have been retained within this area. It was also observed that when this zone was eluted and rechromatographed in the toluene-propylene glycol system for 24 hours it was separated into 3 distinct bands all of which were U.V. absorbing, BT-reactive and C^{14}-labeled and whose polarity was between that of hydrocortisone and corticosterone. Cholesterol-4-C^{14} not utilized during the incubation had either run off the paper or was located at the bottom of the strip. The corticoid area was eluted and aliquots counted at infinite thinness in a gas-flow counter for C^{14}-activity and assayed for blue tetrazolium (BT) chromogens by the method of Nowaczynski *et al.*(9).

Preparation of labeled esters. The cholesterol esters used as tracers in this study were prepared by incubation of cholesterol-4-C^{14} with the appropriate purified fatty acid and the cholesterol esterase system described by Swell and Treadwell(10).

Results. The results presented in Table I show that in the presence of a TPNH generating system a tracer amount of free cholesterol-4-C^{14} was rapidly and efficiently converted to labeled steroid. The incubations carried out in the absence of added cofactors served as controls. All values obtained from these control samples were equal to those from comparable controls inactivated immediately after the tracer was added. Values obtained from the zero hour controls, therefore, are not shown in Table I because they are repetitious.

In Exp. 1, a decrease of 59.5% in C^{14}-activity of the free sterol digitonide fraction occurred when the incubations were carried out with a TPNH generating system. Ninety-three percent of this decrease in C^{14}-activity was accounted for in labeled steroids eluted from the paper chromatograms. At the same time, a 57% decrease in free cholesterol content of the homogenate occurred while the cholesterol ester level remained constant. No conversion of free cholesterol-4-C^{14} to labeled ester was detected.

TABLE I. Incorporation of Cholesterol-4-C^{14} into Corticoids by Hog Adrenal Homogenates.

Exp. No.	Cofactors added	Sterol added	Homogenate cholesterol content (μg/100 mg adrenal)		CPM $\times$ 10^3 recovered as sterol digitonide		CPM $\times$ 10^3 eluted from steroid zone	BT chromogens steroid zone (μg/100 mg adrenal)
			Free	Ester	Free	Ester		
1	None	Chol-4-C^{14}* (195,000 cpm)	324	199	193	0	0	
1	TPN + G6P	*Idem*	140	200	78	0	107	
2	None	Chol-4-C^{14}* (214,000 cpm)	306	356	213	0	0	14
2	TPN + G6P	*Idem*	174	351	103	0	85	77
3	None	Chol-4-C^{14} oleate† (63,000 cpm)	311	637	0	56	0	2
3	TPN + G6P	*Idem*	183	641	0	53	0	50
4	None	Chol-4-C^{14} linoleate‡ (145,000 cpm)	442	480	0	136	0	11
4	TPN + G6P	*Idem*	290	490	0	140	0	97

* 5 μg of cholesterol-4-C^{14} in propylene glycol added per 100 mg adrenal tissue.
† 3.5 μg of cholesterol-4-C^{14} oleate in propylene glycol added per 100 mg adrenal tissue.
‡ 8.6 μg of cholesterol-4-C^{14} linoleate in propylene glycol added per 100 mg adrenal tissue.

Likewise, in Exp. 2, a decrease in C^{14}-activity of 51.5% occurred in the free sterol digitonide fraction when TPN and G6P were added to the homogenates. Seventy-seven percent of these counts were found in the steroid area. A decrease of 43% in free cholesterol content of the homogenates occurred and again the ester level was unchanged. A significant increase in BT chromogens was found in the steroid zone when a TPNH generating system was added to the incubation mixtures. The BT chromogen assay was performed to serve primarily as an index of corticoid production since it measures only those steroids possessing an α-ketol grouping in the side chain.

When C^{14}-labeled cholesterol oleate or linoleate in tracer amounts was incubated in this system under optimal conditions for steroidogenesis, no conversion of the esters to either labeled free cholesterol or labeled steroids occurred. However, significant decreases in free cholesterol content were observed and steroids were produced. The cholesterol ester content of the homogenates remained constant during steroidogenesis.

Discussion. This work has clearly demonstrated that when a tracer dose of free cholesterol-4-C^{14} is incubated with hog adrenal homogenates, under optimal conditions for steroidogenesis, labeled steroids are produced efficiently. In a previous study(1), it was found that exogenous free cholesterol failed to promote steroidogenesis when added to homogenates either at the start of incubation or after 2 hours. However, if pregnenolone is added after 2 hours steroid production is greatly increased. Thus, the systems involved in steroid hydroxylations along the path from pregnenolone to corticoids are still active after a 2-hour incubation. It is possible, of course, that the enzyme system(s) responsible for cleaving the cholesterol side chain is no longer active after 2 hours. However, it is also possible that the endogenous pool of cholesterol used for steroid synthesis is depleted and exogenous cholesterol cannot enter this pool. The failure of cholesterol to promote steroid synthesis when added at the start of incubation would imply inadequate mixing of exogenous and endogenous cholesterol since the enzyme systems from cholesterol to corticoids are active at this time. Therefore, the production of labeled steroids from tracer doses of cholesterol-4-C^{14} reported here may have been the result of exchange between the labeled cholesterol and endogenous cholesterol in the active pool

rather than mixing of exogenous and endogenous cholesterol.

It would also appear that the cholesterol esters in these homogenates are inert. Labeled esters were not synthesized from cholesterol-4-C^{14} and when C^{14}-labeled esters were added to the homogenates, neither the free cholesterol nor the steroids produced during incubation were labeled. It is possible that the labeled esters did not exchange with the endogenous esters and become available as a source of labeled free cholesterol, or that the labeled esters used in this study are not utilized for steroid production although these are the major cholesterol esters of hog adrenal as determined by gas liquid chromatography.† Another possible explanation for this finding is that hog adrenal lacks cholesterol esterase since labeled esters added were not converted to free cholesterol. Thus, any labeled ester present, even if in an active pool, could not be converted to free cholesterol. The constant chemical levels of cholesterol esters in these homogenates lend support to this possibility.

Summary. Tracer amounts of cholesterol-4-C^{14} added to hog adrenal homogenates and incubated under conditions favorable for steroid synthesis were rapidly and efficiently converted to labeled steroids. Addition of cholesterol-4-C^{14} esters, prepared enzymatically and incubated under the same conditions, did not give C^{14}-activity in the steroids produced. Conversion of free cholesterol to cholesterol esters or of esters to free cholesterol as measured by C^{14}-activity of the sterol digitonides did not occur in this system.

† Unpublished findings.

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Measurement of Serum Binding Capacity for Vitamin B_{12}. (27583)

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Several investigators have dealt with the problems of binding of vit B_{12} by serum and serum protein fractions and how "free" and "bound" vit B_{12} are distributed in biological material(1-6).

The determinations of "bound" vit B_{12} have been carried out by different methods, which may account for the varying results obtained. To explain some of the apparent discrepancies we have compared 4 different methods for assaying serum binding of vit B_{12} : dialysis; availability of vit B_{12} for *Lactobacillus leichmannii* and for *Euglena gracilis;* and uptake of vit B_{12} by wild type *Escherichia coli.*

Material. Sera were collected aseptically from 24 healthy adults and stored at –20°C in vials containing 1 ml serum each. The vit B_{12} content of each serum was assayed by the *L. leichmannii* method(7). For the binding experiments mixtures were made up of 0.8 ml serum and 0.2 ml of a sterile solution of vit B_{12} in 0.9% saline, containing 10 mg KCN per liter. This mixture was incubated aseptically at 28°C for 18 hours before assay.

Methods. a. *Dialysis.* The preincubated