Table 1. Effects of tetrodotoxin on the metabolism of slices of rat brain cortex. All tissue was incubated for 1 hour at 37°C in oxygen in a medium consisting of Krebs-Ringer solution that contained phosphate and glucose.

Changes in conditions	No drug added	TTX added (3 μM)
Oxygen consumed (μl per mg dry wt. of tissue per hour)		
None	12.0 ± 0.3	12.5 ± 0.6
Electrical stimulation	15.6 ± .3	12.3 ± .3
Ca^{++} omitted	15.4 ± .1	13.0 ± .3
KCl (100 mM) added	17.0 ± .8	16.6 ± .8
$^{14}CO_2$ evolved (mμg mole per 100 mg wet wt. of tissue per hour)		
Acetate-1-^{14}C added	88 ± 6	82 ± 6
Acetate-1-^{14}C added and with electrical stimulation	51 ± 3	80 ± 4
Acetate-1-^{14}C and KCl (100 mM) added	141 ± 5	139 ± 6

sium-stimulated brain respiration. In this case, the flux of potassium ions, which activate the membrane adenosine triphosphatase and thereby stimulate brain cell respiration, is unimpeded by TTX.

Results obtained by the use of labeled acetate are of importance in connection with these conclusions. The rate of evolution of $^{14}CO_2$ from acetate-1-^{14}C in the presence of slices of rat brain cortex in a Krebs-Ringer medium containing glucose is increased by the addition of potassium ions, and it is a sodium-dependent, ouabain-sensitive process (*10*). In addition, the conversion of acetate to acetyl coenzyme A, a necessary preliminary to acetate oxidation, is stimulated by potassium ions and inhibited by sodium ions (*16*). It follows that the rate of acetate oxidation by the brain cell will be dependent on the operation of two factors: (i) influx of potassium ions or efflux of sodium ions, either of which will increase the rate of conversion of acetate to acetyl coenzyme A, and (ii) the activity of membrane adenosine triphosphatase that will partly control the rate of respiration and that is both sodium- and potassium-dependent. It would be expected that TTX would not affect potassium stimulation of acetate oxidation, as it does not impede the flux of potassium ions. Results given in Table 1 confirm this. However, it might be expected that electrical stimulation, by increasing influx of sodium ions, would suppress acetate oxidation. That such suppression occurs is already known (*17*). The TTX, by its suppression of sodium conductance, should therefore block the inhibitory effect of electrical stimulation on acetate oxidation to CO_2. Results of experiments to test this conclusion are given in Table 1 and show that TTX prevents the fall in the rate of acetate oxidation that occurs upon electrical stimulation.

A variety of experiments, which will be described in detail elsewhere, have shown that TTX has no inhibitory effect on the active transport of amino acids or of phosphate ions into the brain cell. Its mode of action on metabolism, therefore, is very different from that of ouabain, which is also known to suppress electrical stimulation of brain respiration (*18*) but which, in contrast to TTX, antagonizes potassium-stimulated brain respiration (*19*).

Our results show that changes in the cation concentration of the incubation medium, or the application of electrical impulses, bring about concomitant changes in the influx or efflux of sodium and potassium ions into the brain cell with subsequent changes of brain metabolism. Therefore, TTX may be used, with advantage, for the study of those effects on brain metabolism brought about by cationic fluxes at the brain cell membrane.

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Regulation of the Adrenal Cortex Secretory Pattern by Adrenocorticotropin

Abstract. *Adrenal gland homogenates from rabbits stimulated for 28 days with adrenocorticotropin have been shown to synthesize cortisol. The total amount of cortisol formed was the same whether progesterone-4-^{14}C or Δ^5-pregnenolone-4-^{14}C was used as exogenous substrate. However, when Δ^5-pregnenolone substrate was used the specific activity of the isolated cortisol was four times as great as when progesterone was used.*

In 1954 Kass *et al.* (*1*) reported that the adrenal secretory pattern of the rabbit could be altered from primarily corticosterone (B_k) to primarily cortisol (F_k). This change was effected by daily stimulation with 12 to 25 units of porcine adrenocorticotropin (ACTH) for 25- to 28-day periods. Confirmation of the increased F_k secretion after similar stimulation with ACTH has been published by Yudaev and Afinogenova and by Krum and Glenn (*2*). Neither reported that there was more F_k than B_k secreted as originally found by Kass and co-workers (*1*), although the B_k secretion rate decreased in both cases. Yudaev and Morozova (*3*) later reported that results of incubations of adrenal slices from rabbits injected daily with ACTH for 24 days prior to being killed indicated that the increase in F_k with concomitant decrease in B_k secretion rates was due to a partial inhibition of the 21-hydroxylase. This partial inhibition would, according to the authors, make more progesterone available for 17α-hydroxylation. The activity of the 21-hydroxylase enzyme per gram of adrenal tissue was reported to be twice as great in tissue from control animals as in ACTH-stimulated animals. However, since the adrenal weights of experimental animals were approximately twice those of control animals, the apparent "inhibition" of the 21-hydroxylase reported to be effected by ACTH stimulation was actually only a lack of stimulation of this enzyme activity. In the present report results of studies conducted to investigate the mechanism by which ACTH stimulates F_k production by adrenal cortical tissue of the rabbit are presented. The results indicate a possible change in the metabolic pathway of pregnenolone in adrenal tissue from stimulated rabbits.

Table 1. Amount of corticosteroids, determined as their acetate derivatives, formed in vitro per 100 mg of homogenate of adrenal tissue from rabbits prestimulated by daily injection of 25 units (experiment 1) or 40 units (experiment 2) of ACTH for 28 days compared with tissue from nonstimulated control rabbits. Substrate used was either progesterone-4-^{14}C (prog-4-^{14}C) or Δ^5-pregnenolone-4-^{14}C (preg-4-^{14}C). Figures are averages of duplicate incubations in experiment 2, or of 3 to 8 incubations in experiment 1. F_k-Ac, cortisol acetate; B_k-Ac, corticosterone acetate; DOCA, deoxycorticosterone acetate; N.D., none detected.

Substrate and concentration (μmolar)	Corticosteroids formed (μg/100 mg of tissue)						F_k/B_k (Exptl.)
	F_k-Ac		B_k-Ac		DOCA		
	Exptl.	Control	Exptl.	Control	Exptl.	Control	
Experiment 1							
Prog-4-^{14}C (165)	14.2	N.D.	75.4	79.2	N.D.	8.8	0.19
Prog-4-^{14}C (330)	9.5	N.D.	110.2	90.9	N.D.	24.2	0.09
Prog-4-^{14}C (660)	3.8	N.D.	124.7	67.5	38.7	117.2	0.03
Experiment 2							
Prog-4-^{14}C (165)	21.4		38.4		N.D.		0.56
Preg-4-^{14}C (82.5)	19.4	N.D.	26.0	30.2	N.D.	5.6	0.75
Preg-4-^{14}C (165)	22.9	N.D.	36.0	45.7	N.D.	18.2	0.64
Preg-4-^{14}C (330)	33.9	N.D.	72.8	47.7	N.D.	83.7	0.47
Preg-4-^{14}C (660)	30.8	N.D.	120.2	42.6	16.2	165.9	0.26

Male New Zealand white rabbits weighing 2.3 to 3.2 kg were injected daily for 28 days with 25 to 40 units of purified porcine ACTH (*4*) in 0.2 ml of 5 percent beeswax in peanut oil. Control animals were injected with beeswax-peanut oil vehicle only. At the end of the injection period the animals were killed and the adrenals removed. Adrenal glands from three to six similarly treated rabbits were pooled and homogenized in approximately 2 ml of Krebs-Ringer phosphate buffer per 100 mg of tissue. Nicotinamide, nicotinamide-adenine dinucleotide phosphate, nicotinamide-adenine dinucleotide, adenosine triphosphate, sodium fumarate, β-D-glucose, and glucose-6-phosphate were added to the buffer. Substrates were included in the glass-stoppered incubation flasks in 0.1 ml of propylene glycol. Two units of crystalline glucose-6-phosphate dehydrogenase (Sigma type VI) were added to each flask followed by two ml of tissue homogenate. The flasks were gassed with 95 percent O_2 and 5 percent CO_2, stoppered, and placed in a constant-temperature shaking water bath for 3 hours at 37.5° ± 0.5°C. The reactions were terminated by adding 5 ml of organic extractant, dichloromethane–ethyl acetate (1 : 1), mixing thoroughly, and freezing at −20°C. After being thawed the incubation media were extracted with additional organic solvent to recover the steroids. This initial extract was further purified by partitioning between ligroin and 70 percent ethanol. F_k, B_k, and 11-deoxycorticosterone were further purified and isolated by partition paper chromatography of the free compounds and their acetate derivatives (*5*). The isolated steroid acetates were recovered from the final paper chromatogram by elution with 15 ml of ethanol. Cortisol acetate was quantitatively determined by means of the Porter-Silber reaction (*6*), while the amount of corticosterone acetate and deoxycorticosterone acetate were determined by the blue-tetrazolium reaction (*7*). The amount of ^{14}C in the isolated steroid acetates was determined by counting duplicate aliquots of each sample in a liquid scintillation spectrometer. The disintegrations per minute figure was converted to a percentage of the original exogenous ^{14}C-labeled substrate included in the incubation flasks. From the number of micromoles of substrate, the number of micromoles converted to the isolated steroid was calculated. This figure was then divided by the total number of micromoles of the steroid acetate isolated and multiplied by 100 to give the percentage of the isolated steroid that was formed from the exogenous ^{14}C-labeled substrate. All quantitative values were corrected for percent recovery and zero-time control values.

The results of an experiment in which progesterone-4-^{14}C was incubated with homogenates of adrenal glands from rabbits injected with 25 units of ACTH per day for 28 days are shown in Table 1 (experiment 1). All values are averages of from three to eight incubations. It is evident from these data that ACTH-prestimulated tissue synthesized F_k, whereas tissue from control animals did not. However, the F_k/B_k ratio decreased with increasing substrate concentration in experimental tissue incubations. This was due to both a decrease in the amount of F_k and an increase in the amount of B_k formed. At the highest substrate concentration the amount of B_k synthesized per 100 mg of experimental tissue was almost double that formed by control tissue. However, the combined amount of B_k plus 11-deoxycorticosterone was approximately the same for both tissues.

The data in Table 2 (experiment 1) indicate that 40 to 80 percent of the isolated B_k was derived from the progesterone-4-^{14}C substrate in both experimental and control tissue incubations, while only 1 to 14 percent of the F_k formed by experimental tissue came from this substrate. The percentages increased directly with substrate concentration. The fact that progesterone appeared to be a less efficient precursor for F_k than for B_k synthesis led us to do similar incubations in which either progesterone-4-^{14}C or Δ^5-pregnenolone-4-^{14}C substrates were used. In these experiments the animals were injected with more ACTH per day

Table 2. Percentage of isolated corticosteroids which were formed from the exogenous substrate, either pregnenolone- or progesterone-4-^{14}C. Other details as in Table 1 and text.

Substrate and concentration (μmolar)	Corticosteroids formed (% from added substrate)					
	F_kAc		B_k-Ac		DOCA	
	Exptl.	Control	Exptl.	Control	Exptl.	Control
Experiment 1						
Prog-4-^{14}C (165)	1.5	N.D.	42.4	51.5	N.D.	45.7
Prog-4-^{14}C (330)	9.1	N.D.	60.8	74.6	N.D.	57.2
Prog-4-^{14}C (660)	14.1	N.D.	74.8	83.1	72.2	90.0
Experiment 2						
Prog-4-^{14}C (165)	13.3		73.0		N.D.	
Preg-4-^{14}C (82.5)	34.3	N.D.	47.6	59.5	N.D.	N.D.
Preg-4-^{14}C (165)	53.8	N.D.	91.8	81.9	N.D.	35.1
Preg-4-^{14}C (330)	74.7	N.D.	95.6	88.1	N.D.	91.2
Preg-4-^{14}C (660)	96.8	N.D.	100.	100.	27.0	95.6

(40 units) than in the previous experiment.

Representative results from these experiments are shown in Tables 1 and 2 (experiment 2). All values are averages of duplicate incubations. It is evident from these data that the experimental tissue again synthesized F_k and the control tissue did not. The total amount of F_k synthesized was independent of the substrate used. However, the percentage of the total which was formed from the added Δ^5-pregnenolone-4-^{14}C was approximately four times as great as the percentage of the total formed from the added progesterone-4-^{14}C (Table 2, experiment 2), when both substrates were used at a 165 μmolar concentration.

The percentage of the total isolated B_k synthesized from the substrates ranged from 50 to 100 percent, regardless of the tissue source or substrate. The percentage of the F_k formed from Δ^5-pregnenolone-4-^{14}C at 165 μmolar concentration by tissue from animals injected with 40 units of ACTH was only about half that of the B_k isolated from the same incubation media, however. Results of experiments with ½, 2, and 4 times this substrate concentration also showed a smaller percentage of F_k than B_k to be formed from the ^{14}C-labeled substrate. The F_k/B_k ratio varied inversely with substrate concentration. This was due primarily to increases in the amount of B_k formed (Table 1). The amount of B_k synthesized per 100 mg of experimental tissue again exceeded the amount synthesized by control tissue at the two higher substrate concentrations. As in the experiments with progesterone-4-^{14}C substrate, however, the total of 11-deoxycorticosterone plus B_k formed per 100 mg of adrenal tissue from control animals was at least equal to the corresponding figure from experimental tissue incubations.

The data are consistent with the explanation that ACTH stimulation caused the appearance of a 17α-hydroxylase enzyme activity which has a relative specificity for Δ^5-pregnenolone over progesterone. This would explain the observation that Δ^5-pregnenolone-4-^{14}C is a better precursor of F_k than is progesterone-4-^{14}C. A relatively large quantity of exogenous progesterone-4-^{14}C substrate could inhibit the more efficient conversion of endogenous Δ^5-pregnenolone to F_k via 17α-hydroxypregnenolone. Thus, the decrease in total F_k production with increasing progesterone-4-^{14}C substrate concentration supports the postulate of the existence of a Δ^5-pregnenolone-17α-hydroxylase pathway for F_k formation. Due primarily to an increase in the B_k formed, the F_k/B_k ratio increased with decreasing Δ^5-pregnenolone-4-^{14}C substrate concentration. This indicates that the 17α-hydroxylase activity is responsible for an increasing percentage of the Δ^5-pregnenolone metabolism at the lower concentrations of this substrate. At even lower Δ^5-pregnenolone concentrations than used in these experiments the F_k production might exceed B_k production. This could explain the original observations of Kass and co-workers of an F_k/B_k ratio of greater than 1 in adrenal vein blood of similarly stimulated rabbits. The data showing that a somewhat lower percentage of the F_k than of the B_k was formed from the exogenous Δ^5-pregnenolone-4-^{14}C substrate is interpreted to mean that after the large amounts of the exogenous labeled substrate were metabolized, a relatively greater percentage of nonlabeled, endogenous Δ^5-pregnenolone was metabolized to F_k than to B_k. The fact that no ^{14}C-labeled Δ^5-pregnenolone remained at the end of the 3-hour incubation period supports this interpretation.

We have found very little evidence supporting an inhibition of the 21-hydroxylase in adrenal tissue from ACTH-stimulated rabbits. The total production of the major 21-hydroxylated steroids tended to be slightly lower per 100 mg of ACTH-stimulated adrenal tissue. As noted earlier, however, the adrenal weights of the ACTH animals approach twice those of control animals.

I feel that the evidence presented supports the hypothesis originally suggested by Kass *et al.* (*1*), that prolonged stimulation of rabbits by ACTH results in an increased adrenal 17α-hydroxylase activity. It now appears that this enzyme activity has a substrate specificity favoring 17α-hydroxylation of Δ^5-pregnenolone rather than progesterone.

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Light Chains of Rabbit Immunoglobulin: Assignment to the Kappa Class

Abstract. *Normal rabbit gamma globulin was reduced under conditions presumed to break only interchain disulfide bridges, and the reduced product was then blocked with C^{14}-iodoacetamide. The light chains were separated from the heavy chains and subjected to peptic digestion. Two radioactive peptides were recovered from the digest. The peptides are apparently overlapping and represent the carboxy-terminus. Comparison of this region in the rabbit light chains with the corresponding amino acid sequences in various mouse and human light chains indicates that the rabbit light chains are of the κ-class.*

Human immunoglobulin light chains fall into two distinct immunologic classes currently designated κ and λ types. Studies of amino acid sequences indicate that each of these classes has a unique amino acid sequence for that half of the polypeptide chain which extends to the carboxy-terminus (*1*). This region includes the cysteine residue contributing to the interchain disulfide bridge that links heavy and light chains (*2*). The other halves of these light chains, the amino-terminal portions, are variable within a class and presumably represent the regions of light chains which contribute to the specificity of antibodies.

Comparative studies of the amino acid sequences of light chains from human and mouse Bence Jones proteins resulted in the discovery that the variable amino-terminal regions of these two species were virtually indistinguishable, whereas their carboxy-terminal halves differed in what might be called the "normal" or "expected" amount on the basis of comparative sequence studies on other proteins like hemo-