(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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31 March 1967

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-

Table 1. Amino acid analyses of peptides P-I and P-II.

Substance	Amount recovered (nmole)		Residues per mole	
	I	II	I	II
Ammonia	94.8	97.4		
Arginine	41.5	35.4	0.9	0.9
Carboxymethyl-cysteine	40.7	29.9	0.9	0.8
Aspartic acid	92.2	79.4	2.0	2.1
Serine	2.6	18.8	0.1	0.5
Glycine	49.6	42.1	1.1	1.1
Phenylalanine	0	35.9	0	0.9

globin (*3*). On the other hand, the sequence identities were enough to warrant assigning those mouse Bence Jones light chains studied to the κ class. The indistinguishability of mouse and human light chain variable regions was interpreted as favoring Dreyer and Bennett's model of an immune system in which all possible immunoglobulin amino acid sequences are delineated in the nucleotide sequences of the organism's germ cell DNA (*4*). The fact that not all species had immunoglobulin light chains that fell into such an indistinguishable set prompted an immediate challenge to such an interpretation (*5*). The observed sequence differences between rabbit light chains and human and mouse light chains, for example, could most readily be explained if only one, or a small number, of light chain genes existed. It seemed highly unlikely that the same amino acid replacements would have occurred in all the thousands of postulated genes. On the other hand, the possibility was presented that rabbit light chains might be made up of a class completely different from either κ or λ types (*6*). For this reason, we studied the constant portions of rabbit immunoglobulin light chains to determine their correspondences and differences when compared with mouse and human light chains in those regions.

The peptide or peptides involved in linking the light chains to the heavy chains were isolated by reducing normal rabbit γ-globulin (Pentex) with dithiothreitol (*7*). The sulfhydryl groups liberated by reduction of the interchain disulfide bridges were then blocked with C^{14}-iodoacetamide (*8*). Light chains were separated from heavy chains on Sephadex G-100 in 1*M* propionic acid (*9*); the light chains were all of allotype 4 (*10*). After treatment with carboxypeptidase A (*8*) for 1 hour, half the radioactivity was found in carboxyamidemethylcysteine. The light chains were only slightly soluble in the ammonium bicarbonate buffer, however, and a greater percentage might have been released if the system were homogeneous. Thus the bulk of the radioactivity was situated at the carboxy-terminus. Other preparations of light chain were digested with pepsin under conditions that yield peptides of significant length in the cases of both κ- and λ-chains from human normal γ-globulin (*2*). The peptic digests were dried, dissolved in 0.05*M* ammonium bicarbonate and passed over a Sephadex G-25 column (Fig. 1). About 70 percent of the radioactivity appeared in a single retarded peak. Furthermore, the remainder of the radioactivity could be converted into a similarly retarded fraction upon further peptic digestion. The fraction obtained from gel filtration was pooled, dried, and applied to a Dowex 50X2 column (Fig. 2). Two radioactive peaks were obtained, one eluting at *p*H 6.6 and the other at 7.9. The two peaks (P-I and P-II) were isolated and subjected to preparative paper electrophoresis. In each case, only a single ninhydrin-positive band was obtained. Furthermore, in both cases all the radioactivity migrated with the band. The peptides were eluted, and their amino acid compositions were determined on a Spinco amino acid analyzer (Table 1). The peptide found in higher yield, P-I, was a pentapeptide. The other major fragment, P-II, contained the same five amino acids as P-I, plus one residue of phenylalanine and half a residue of serine. The fractional serine value was obtained repeatedly, in spite of the substantial amounts of peptide processed. Our tentative interpretation is that P-II is really a mixture of two peptides, one having a serine on its

Table 2. Carboxy-terminals of various immunoglobulin G light chains

	8	7	6	5	4	3	2	1	1a
Human λ	..	Lys–	Thr–	Val–	Ala–	Pro–	Thr–	Gly–	Cys–Ser
Human κ	..	Lys–	Ser–	Phe–	Asn–	Arg–	Gly–	Glu–	Cys
Mouse κ	..	Lys–	Ser–	Phe–	Asn–	Arg–	Asn–	Glu–	Cys
Rabbit L	.....	Ser–	Phe–	Asn–	Arg	(Gly,Asp)			Cys

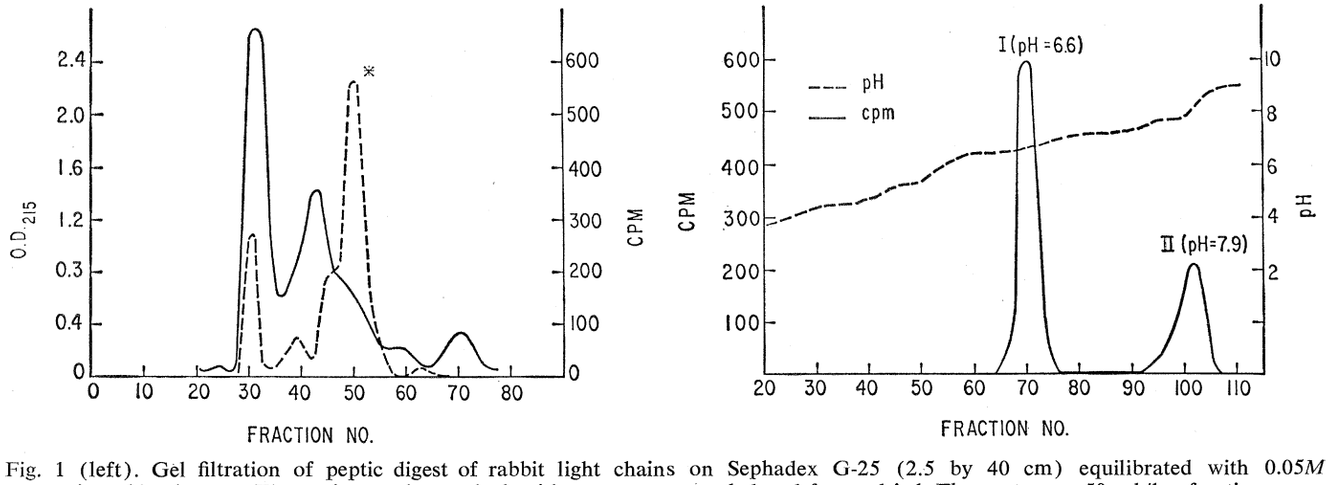

Fig. 1 (left). Gel filtration of peptic digest of rabbit light chains on Sephadex G-25 (2.5 by 40 cm) equilibrated with 0.05*M* ammonium bicarbonate. The major peak, marked with a star, was pooled and freeze-dried. Flow rate was 50 ml/hr; fractions were 4.0 ml each. Solid line is O.D.$_{215}$ (optical density); broken line is radioactivity (*CPM*, counts per minute). Fig. 2 (right). Chromatography on Dowex 50X2 (1 by 15 cm) of the major radioactive peak isolated by gel filtration. Fractions were 3.0 ml each; flow rate was 60 ml/hr. Solid line, radioactivity; broken line, *p*H.

amino-terminal, and the other, lacking serine, being a further peptic digestion product of the first.

Sustained peptic digestion converted most of P-II into P-I. Tryptic digestion of P-I yielded two peptides, asparaginylarginine and a tripeptide containing glycine, aspartic acid, and carboxyamidemethylcysteine. An amino acid sequence based on these data is proposed (Table 2) and compared with the known sequences of mouse and human κ-chains and the human λ-chain. The rabbit has six of seven residues in common with the human κ-chain and five of seven in common with the mouse κ-chain. The mouse and human κ-chains have six of seven residues in common in this region. On the other hand, the human λ sequence is distinctly different (Table 2).

In that, after sustained peptic digestion, more than 80 percent of the radioactivity incorporated into rabbit light chains during interchain cleavage could be recovered as peptides I and II, the bulk of rabbit light chains are of the κ variety. Why the rabbit carboxy-terminal regions are so similar to those of mouse and human κ-chains, whereas the amino-terminal regions are distinctly different, is not at all clear. In the meantime, we maintain that the distinctive amino acid sequences are most readily understood in terms of a κ cistron from a single germ line coding for the variable regions of κ-chains.

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27 March 1967

Thermoregulation in the Desert Iguana Dipsosaurus dorsalis

Abstract. *The body temperature of desert iguanas implanted with miniature temperature-sensitive radio transmitters was continuously monitored in their natural habitat. Extensive thermoregulatory behavior occurred in retreat burrows prior to morning emergence. Such behavior permits the iguana to emerge from below ground at its preferred body temperature rather than suboptimal temperature at which activity in the burrow is initiated.*

Nearly all studies of body temperatures of reptiles have been based on the thermal categories set forth by Cowles and Bogert (*1*). Such designations as mean preferred temperature and lethal temperature are usually compiled from cloacal temperatures taken with a quick-recording mercury thermometer; although these categories are valuable in presenting a comparative picture of interspecific thermal requirements, they reveal little of the dynamics of daily thermoregulation.

Recently Mackay (*2*) obtained continuous recordings of deep-body temperatures of free-living Galápagos tortoises and marine iguanas by use of biotelemetry. The green iguana was similarly studied (*3*). In both studies miniature temperature-sensitive radio transmitters located within the body cavity proved far superior to mercury thermometers or thermocouple potentiometers in documenting temperature regulation in that the animals were unrestrained and undisturbed.

Our purpose was to explore in detail, by use of telemetry, regulation of temperature in the desert iguana *Dipsosaurus dorsalis*, a species previously studied with conventional techniques (*4*), in Tahquitz Canyon, Palm Springs, California, during the last week of May 1966. An enclosure (10 by 7 m, with walls 1 m high) was constructed of corrugated cardboard around a section of desert floor containing several burrows of kangaroo rats, which desert iguanas prefer for retreat (*4*). Several creosote bushes within the enclosure shaded about one-fourth of it. The floor carried an antenna grid comprising 25 loops of 14-gauge copper wire; they were 10 m long, 0.25 m wide, and spaced 0.1 m apart. Each loop was connected to a switchboard with coaxial cable so that signals could be recorded by way of any one antenna or any combination. Thus one could monitor each animal individually except when infrequently two lizards positioned themselves on the same antenna transect; and one could record movements and body temperatures of lizards underground to a depth of 0.25 m. Lizards that climbed the creosote bushes were received to a height of 0.4 m.

The tails of four male desert iguanas, captured within 100 m of the enclosure, were banded with colored paint for identification. Through a paraasagittal incision 1 cm long just anterior to the left hind leg, a thermal transmitter (*5*) was inserted in the body cavity. Since the telemeter (1 by 2 cm, 1 g) was about two-thirds the size of an iguana's egg, we felt that it would not cause discomfort to a male lizard. The incision was tightly sutured with nylon thread, and the animals were held for several hours to see that no hemorrhaging occurred. Implanted lizards were released into the enclosure during the late afternoon of the day of capture. Two unoperated animals also were released as behavioral controls; there was no difference in their behavior.

Temperature recordings and behavioral observations were made on all surface-dwelling lizards from a blind

Table 1. Selected deep-body temperatures of four desert iguanas implanted with thermal transmitters; all but the last item were recorded in a natural habitat. Numbers of temperatures appear in parentheses.

Time, conditions	Body temperature (°C)	
	Mean	Range
Emergence from burrow (17)	38.9	35.5–40.0
Retreat to burrow (16)	39.2	35.0–42.0
Initiation of morning activity in burrow (16)	31.0	28.0–34.0
Cessation of afternoon activity in burrow (14)	36.0	32.5–37.2
Preference throughout 2 days (40)	38.0	35.2–45.7
Preference throughout 2 days in laboratory gradient (40)	39.0	35.7–44.0