sibly its dissociation from methionine, at the inner surface of membrane, in agreement with ALVARADO's hypothesis[10]. It can also be assumed that sugars act on the coupling between energy supplying mechanisms and transport, probably regulating it. The approach of this aspect of the problem, concerning hormonal action upon this process, is object to further investigation.

Zusammenfassung. Bei mit Zucker aufgeladenen Ehrlich-Ascites-Tumorzellen steigert sich das Eindringen des Methionins in die Zelle. Wenn sich dieselben Zucker im extrazellulären Raum befinden, bleibt dieser Effekt aus. Die möglichen Ursachen dieses Effektes werden besprochen.

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1-^{14}C-Acetyl-Coenzyme A as Steroid Precursor in the Monkey Adrenal in vitro

^{14}C-Activity from sodium 1-^{14}C-acetate has been shown to be incorporated into steroids by various endocrine tissues[1]. The present paper reports the isolation of ^{3}H/^{14}C double labelled pregnenolone (3β-hydroxy-pregn-5-ene-20-one), DHEA (dehydro-epiandrosterone = 3β-hydroxy-androst-5-ene-17-one) and progesterone (pregn-4-ene-3,20-dione) after in vitro incubation of monkey adrenal slices with 1-^{14}C-acetyl-Coenzyme A and ^{3}H-pregnenolone. Evidence is presented of different pathways for the biosynthesis of pregnenolone and DHEA.

Methods. Fresh adrenal slices (0.5 g; 0.5 mm thickness) from a Rhesus monkey were incubated[2] in 4 ml of Krebs-Ringer[3] phosphate buffer (pH 7.4) and 2 ml of heparinized blood plasma from the same monkey. The following substances were added: 50 μC of 1-^{14}C-acetyl-CoA (1.07 μM; 111×10^6 dpm; purchased from Tracerlab Inc., Waltham, Mass., USA); 1.5 μC of 7α-^{3}H-pregnenolone (0.0001 μM; 3.3×10^6 dpm; New England Nuclear, Boston, Mass.); 0.2 ml of propylene glycol; 8 mg glucose and 50,000 IU potassium penicillin G. After 6 h the tissue was homogenized in a virtis homogenizer. Free steroids were extracted with ether, free sterols with *n*-hexane. The isolation of free neutral steroids is described in detail elsewhere[2]. It included protein precipitation, purification by solvent partitions, fourfold thin layer or paper chromatography respectively and formation of acetates[4] and 2,4-dinitrophenylhydrazones[5]. To each of the fractions of pregnenolone, DHEA and progesterone 15 mg of standard steroids were added and twice crystallized from aqueous ethanol to constant specific activity (Table I).

Results and discussion. In the hexane extract containing most of the presterols, the free sterols and a few per cent of free steroids[6], 32,900 dpm ^{3}H and 4,350,000 dpm ^{14}C were found. The compounds of this fraction were not further characterized.

Table I contains the specific activities of pregnenolone, DHEA and progesterone after addition of 15 mg standards and twofold crystallization. Table II shows the distribution of total ^{3}H and ^{14}C activity in different fractions of the ether extract.

Acetyl-coenzyme A was isolated as an intermediate in the conversion of acetate to presterols and to cholesterol[1]. The present data show that the monkey adrenal in vitro can synthesize ^{14}C labelled pregnenolone, DHEA and progesterone from 1-^{14}C-acetyl-CoA also. Further results prove that acetyl-CoA is a preferred precursor over acetate under experimental conditions. This will be published later.

Table I. Specific activity (dpm/mg) of pregnenolone, DHEA and progesterone after chromatography in specified systems, addition of 15 mg standards and twofold crystallization from aqueous ethanol

Steroid	ML[a] I ^{3}H	^{14}C (R[b])	Cr[a] I ^{3}H	^{14}C (R)	ML II ^{3}H	^{14}C (R)	Cr II ^{3}H	^{14}C (R)
Pregnenolone	19,600	71 (277)	18,700	76 (254)	19,900	74 (268)	19,100	76 (250)
DHEA	3,680	61 (6.01)	3,200	58 (5.50)	3,400	67 (5.10)	3,100	62 (5.03)
Progesterone	7,300	182 (40.0)	6,700	95 (75.1)	6,550	75 (87.00)	6,600	71 (92.8)

[a] ML, mother liquor; Cr, crystals. [b] R = dpm ^{3}H/dpm ^{14}C.

Table II. Total ^{3}H and ^{14}C activity (dpm) in different fractions of free neutral steroids as calculated to 100% recovery

Fraction	^{3}H	^{14}C	^{3}H/^{14}C (dpm/dpm)
Pregnenolone	588,000	2,350	250.00
DHEA	111,000	22,000	5.03
Progesterone	212,000	2,280	92.80
X-P-1[a]	161,000	15,300	10.50
X-P-2[a]	358,000	133,000	2.69

[a] 2 fractions more polar than DHEA, which were not further characterized.

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In the present experiment ^{3}H-pregnenolone along with the acetyl-CoA was added to the incubation medium. Pregnenolone is generally considered to be a necessary intermediate in the conversion of acetate via cholesterol to progesterone. Pregnenolone however had a $^{3}H/^{14}C$ ratio higher than progesterone. This may indicate that during the incubation period only part of extracellular ^{3}H-pregnenolone had reached the steroid converting enzymes. By the extraction procedure intracellular pregnenolone was mixed with extracellular ^{3}H-pregnenolone. But progesterone had a $^{3}H/^{14}C$ ratio higher than DHEA. If both metabolites were derived from the same precursor – intracellular pregnenolone – they should have exhibited the same $^{3}H/^{14}$ ratio. The higher ^{14}C content of DHEA suggests ^{14}C-precursors other than pregnenolone for DHEA. A direct conversion of cholesterol into DHEA via 17,20α-dihydroxy-cholesterol[7] and via 17α-hydroxy-pregnenolone[8–10] has been demonstrated by other investigators[11].

Zusammenfassung. Nach In-vitro-Inkubation von Nebennierenschnitten eines Rhesusaffen mit 1-^{14}C-acetyl-CoA und 7α-^{3}H-Pregnenolon konnte man Pregnenolon, DHEA und Progesteron isolieren, die alle sowohl ^{3}H- als auch ^{14}C-Aktivität enthielten. Es ergaben sich Hinweise für verschiedene Biosynthesewege für Pregnenolon und DHEA.

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Some Effects of Laser upon the Bones

The biological effects of laser upon various body tissues are studied with increasing interest, because of the broader use of laser in clinical practice. Up to the present time, little attention was paid to the influences of laser pulsations on bones. In the scanty reports available, some such observations are mentioned. Perforations or excavations of bones are understandable after transmissions of high-power laser pulses[1–3], but according to some reports, also much lower intensities (10–50 J) were sufficient to cause char of bone periosteum with necrotic areas of bone tissues beneath it[4]. But a surprisingly normal macroscopic appearance of bones was reported even after higher intensities of laser radiations[5,6]. Some initial degrees of damage can hardly be found in the bones macroscopically and sometimes not even microscopically. When investigating the influences of various other physical agents on the skeleton[7], we noticed the usefulness of isotopic methods with ^{45}Ca as a tracer, in detecting unconspicuous aberrations in the bone tissue. Therefore, we tried to investigate the influence of laser pulses on the bones in this manner.

Male Wistar rats, weighing 120–130 g, were subjected to 3 laser pulses from a CO_2-laser, working in the IR-spectral area with $\lambda = 10.6$ μm. The 4 mm broad beam reached the area of the knee of the right extremity in expositions, lasting 3 sec each, with 3 sec long intervals. The whole amount of energy applied to the skin surface was estimated to be 9 J. Non-irradiated controls and irradiated animals were kept under the same conditions. After 4, 11, 18, 33, 46, 60, 74 and 102 days post irradiation, groups of 5 animals from each category were formed (i.e. irradiated and non-irradiated). Each animal was given 20 μCi $^{45}CaCl_2$ in 1.0 ml saline i.p. After 48 h the animals were killed by ether narcosis and both tibiae were liberated from surrounding tissues. The bones were investigated chemically for the content of calcium, and dosimetrically for their content of ^{45}Ca in the same manner as reported previously[7,8]. The 48-h uptake was expressed as average of all 5 homologous bone samples.

Although there was no significant difference in the weight of dried bone substance of the tibia (Figure 1) in irradiated and non-irradiated animals, the uptake of radioactive calcium was different (Figure 2). During the first 3 weeks, there were less noticeable differences in this uptake; after this time distinct deviations of the tracer uptake started in the irradiated as well as the non-irradiated legs of experimental animals. The appearance of the irradiated skin and the gross appearance of the bones during preparation were normal. The Table gives a summary of statistical significances (t-test) of values, expressed in Figure 2.

Like various other physical agents (e.g. X-rays, electric current, heat, cold, ultrasound)[7], laser radiations caused disturbances not only in the calcium metabolism in directly irradiated bones, but even in the opposite legs. This was true in spite of the fact that the skin burns healed without complications during the first week after irradia-

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