regular pellets. It was found that CO_2 production was increased somewhat by feeding either the crushed pellets (11.2 μl CO_2 ± 5.9 S.D., in 14 experiments) or milk-soaked bread (13.2 μl CO_2 ± 3.2 S.D., in 6 experiments). There was no statistical difference between groups Ib and Ic ($\alpha = 0.5$) and these are therefore considered as one group of 20 experiments.

It can be seen that CO_2 production increased directly with weight up to 90 g. Using KRAMER's multiple range test[8], group III values were found to be significantly higher than groups I or IV, and group IV significantly greater than group I ($\alpha = 0.01$).

Discussion. The reason for the poor performance in the youngest rats is not known, but it is possible that the results were influenced by increased levels of growth hormone which might be present at this age. Although WINEGRAD et al.[9] demonstrated enhanced CO_2 production when growth hormone was added in vitro, GOODMAN[10] showed that injection of the hormone into rats for 4 days prior to sacrifice resulted in reduced CO_2 production as well as decreased fatty acid synthesis in the isolated fat pad. ALTSCHULER et al.[11] suggested that the increased fatty acid release in their immature rats might also be secondary to the effects of the growth hormone. Unfortunately, no data concerning the levels of growth hormone in rats at various ages are available at present.

We have no explanation for the increased CO_2 production in group III (75–90 g) as compared to the adult group. Neither could HAGEN et al.[2] fully explain the poorer response seen in older rats (over 250 g), but suggested that 'dietary or hormonal' influences were involved. ALTSCHULER et. al.[11] found a decreased tissue nitrogen content in mature rats compared to animals weighing less than 100 g, coincident with reduced activity.

Carbon dioxide production from adipose tissue in rats of various weights (results expressed as μl CO_2 evolved per 100 mg wet tissue/h)

Group	Weight (g)	Mean ± S.D.	No. of experiments
I (b and c)	35– 50	11.8 ± 5.2	20
II	55– 70	17.4 ± 10.4	25
III	75– 90	34.7 ± 9.6	22
IV	150–200	22.7 ± 7.4	10

Zusammenfassung. Es wurde der Einfluss von Insulin auf die CO_2-Produktion im Fettgewebe unreifer Ratten mit einem Gewicht zwischen 35 und 90 g manometrisch bestimmt, wobei mit zunehmendem Gewicht bis zu 90 g eine vermehrte CO_2-Produktion festgestellt werden konnte.

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Identification of Steroid Hormones from *Lacerta sicula* Testes

The occurrence of sex hormones in the gonadal tissue of some representatives of lower vertebrates has been reported in recent years. The analyses of the testicular tissue of marine vertebrates, namely the teleosts *Salmo irideus* and *Cyprinus carpio*[1], *Morone labrax*[2], *Oncorhynchus nerka*[3], and the elasmobranch *Scyliorhinus stellaris*[4] have shown the presence of well-known steroid hormones. Testes and Bidder's organs of *Bufo vulgaris*[5] also produce androgen precursors and oestrogens. Information regarding the androgenic material in *Sauropsida* is still lacking; therefore we have analysed the testicular tissue of the lizard *Lacerta sicula*.

From 400 mature animals, 35 g of material were obtained. The tissue was lyophilized and the free steroids extracted with organic solvents. The conjugated steroids were extracted after acid hydrolysis with HCl and dioxane. The phenolic steroids were separated from the neutral ones by extraction with NaOH. Neutral and phenolic fractions were purified by column and thin-layer chromatography. The identification of the steroids was obtained by means of UV-spectra, characterization of derivatives, and gas-liquid chromatography.

Neutral fraction. Both free and conjugated neutral fractions were purified on neutral alumina column, eluted with mixtures of petroleum ether-ethylacetate. The fractions were then examined on thin-layer, using silica gel G as adsorbent and the mixture acetone/chloroform (5:95) as solvent system. 4 spots were detected in the free neutral extract by exposure to iodine vapour, corresponding to the Rf values of progesterone, androstenedione, androsterone and testosterone. The UV-spectra of the first 2 spots showed maximum absorption at 240 nm in absolute ethanol and 290 nm in concentrated sulphuric acid. Gas-liquid chromatography of the eluates from thin-layer chromatography, using a Barber Colman apparatus with column packed with SE-30 2% in Chromosorb W and an argon flow of 81 ml/min, confirmed the presence of progesterone in the first spot and of androstenedione in the second. By these methods it was also possible to detect 20β-hydroxypregn-4-en-3-one in the first spot and dehydroepiandrosterone and pregnenolone in the second.

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The presence of androsterone in the third spot was confirmed by gas-liquid chromatography and by the Zimmermann reaction. These steroids were present in the tissue in the following concentrations, calculated from the area under the curve in gas-liquid chromatography: progesterone 34.3 μg/kg, 20β-hydroxypregn-4-en-3-one 4.5 μg/kg, androstenedione 53.7 μg/kg, pregnenolone 123.4 μg/kg, dehydroepiandrosterone 454 μg/kg, androsterone 99 μg/kg.

The fourth spot had a very strong UV-absorption maximum at 240 nm in absolute ethanol, but no testosterone could be detected in gas-liquid chromatography. One spot was detected in the conjugated fraction with Rf corresponding to testosterone. The UV-spectrum in ethanol showed an absorption maximum at 240 nm; the oxidation product showed the same Rf of androstenedione on thin-layer chromatography; finally the gas-liquid chromatographic analysis confirmed the presence of testosterone at a concentration of 91 μg/kg.

Phenolic fraction. Both free and conjugated fractions were examined for the presence of oestradiol-17β, oestrone and oestriol. The thin-layer chromatography system used was ethanol-benzene 10:90. The spots corresponding to the 3 oestrogens were eluted, and the UV-spectra in NaOH 0.5 *N* in 80% ethanol were taken. Part of the extract was used for gas-liquid chromatography. By these methods it was possible to detect oestradiol-17β in the free fraction in a concentration of 127 μg/kg and oestrone in both the free and the conjugated fractions in total concentration of 326 μg/kg.

The present investigation has demonstrated that androgenic as well as oestrogenic substances are present in lizard testicular tissue. Testosterone has been shown to be present in the conjugated fraction only. Of particular interest is the ratio progesterone/androstenedione/testosterone, which approaches that of mammals. In fact, in the other lower vertebrates so far examined, androgen precursors are present in a concentration higher than that of testosterone; for instance in *Scyliorhinus stellaris* testes the ratio of these steroids is 100:70:50 (μg/kg)[4], while in *Lacerta sicula* testes it is 34.3:53.7:91 (μg/kg)[6].

Riassunto. Gli autori hanno analizzato gli steroidi presenti nel tessuto testicolare di *Lacerta sicula*. Sono stati identificati i seguenti steroidi: progesterone, androstenedione, testosterone, androsterone, deidroepiandrosterone, pregnenolone, 20β-idrossipregn-4-ene-3-one, estradiolo-17β, estrone. Viene discusso il rapporto progesterone androstenedione testosterone, paragonandolo a quello trovato in altri vertebrati inferiori.

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PRO EXPERIMENTIS

An Apparatus for Direct X-Ray Cinematography Exemplified by Analysis of Some Respiratory Movements in *Gasterosteus aculeatus*

Measurement of movement between elements within intact organisms poses an important problem for functional anatomists. It is always preferable to make such observations in undisturbed animals; often this is absolutely essential. One of the many techniques successfully applied is X-ray cinematography, utilizing an image intensifier[1-3]. However, this technique has limitations in terms of the distinctness of image detail obtainable by present methods.

Direct application of X-rays provides markedly increased resolution, but it can hardly be used in human radiology because of the high dosages required[3]. Since the animals used for these experiments have much thinner bones and much greater tolerance, the dosage required may be permissible. The apparatus, which will be described below, allows one to obtain a rapid sequence of direct X-ray exposures of a small moving object. The results obtained are demonstrated by the analysis of the respiratory movement of 2 cranial elements of the 3-spined stickleback, *Gasterosteus aculeatus*.

X-ray motion pictures of the movements of bony elements may be obtained by the use of two comparable techniques. The indirect technique, applied increasingly in medical diagnostics, utilizes an image intensifier. X-rays impact on a screen covered by a glass tube. Behind the screen is placed a photocathode which releases electrons in an amount depending on the intensity of the X-rays. The electrons are concentrated on and accelerated to a second small screen, which transduces the image to the visible region. It is then observed by means of an optical system, or a television camera, or filmed with a cine-camera. The intensification of brightness of the image on the second screen permits the use of normal blue sensitive films with low kilovoltage and low tube current, thus small X-ray dosage.

The limitation of this technique is due to its indirectness. In spite of their fine grain material, the 2 screens cause an unsharp image. Moreover, an important additional unsharpness is caused by different kinds of 'noise'. First there is considerable variation in the X-ray quanta falling on the first screen, secondly there is directional variation during the emission of electrons. This type of unsharpness can include indistinctiveness and loss of

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