17(4|5]-imidazolyl)-4-androsten-3-one. Activity was apparently reduced by methylation of the imidazole ring of the former and by 11-oxidation or 17-dehydroxylation of the latter.

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## Regeneration of Liver in Lysine-Deficient, Partially Hepatectomized Rats.* (23825)

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Coincident with starvation or maintenance on nitrogen-free diet, there occurs a rapid and extensive loss of nitrogen from liver of rats (1,2). Upon realimentation with protein of adequate nutritional quality, the nitrogen content of liver returns to normal levels in a few days. This accumulation of nitrogen during realimentation of protein-depleted rats has been used for nutritional evaluation of proteins(1,3), of amino acid mixtures and of protein hydrolyzates(4). Similarly, after partial hepatectomy of rats, proliferation of the remaining liver occurs to some extent when they are post-operatively starved(5) or fed protein-free diet(6), and progressively more so as the diet furnishes increased amounts of proteins(6) of adequate nutritional quality(7).

It has been clearly demonstrated that adult rats fed a lysine-deficient amino acid mixture incur loss of appetite and body weight(8), negative nitrogen balance(9) and marked decrease of total circulating serum protein(10). In view of the importance of liver for formation of plasma proteins(11), abnormalities in composition of liver induced by imbalance of dietary amino acids or proteins(12) and increasing evidence that lysine can be a limiting factor in the nutritive value of some diets (13), it was of interest to investigate the effect of lysine deficiency on regeneration of liver.

*Methods.* Mature female rats of hooded strain, maintained in our colony for many years, weighing initially about 200 g were housed individually in cages with screen bottoms. They had access to food and water *ad libitum*. Records were kept of food consumption and each animal received a daily allotment of the ration (Table I) estimated to exceed slightly the

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TABLE I. Composition of Rations.

| Component | Complete ration (g) |
|---|---|
| Amino acid mixture* | 223.2 |
| Suerose and B vit. mixture† | 20. |
| Salt mixture† | 40. |
| Wheat germ oil | 20. |
| Corn oil + vit. A and $D_3$† | 10. |
| Suerose | 686.8 |
| | 1000. |

* The mixture for the complete ration contained: L-arginine hydrochloride 12.96, L-histidine hydrochloride 4.75, L-lysine hydrochloride 13.79, L-tyrosine 6.56, DL-tryptophan 5.12, DL-phenylalanine 18.56, L-cystine 3.68, DL-methionine 10.58, DL-threonine 16.00, L-leucine 15.04, DL-isoleucine 22.08, DL-valine 23.68, L-glutamic acid 29.44, DL-aspartic acid 26.24, glycine 14.72 g. In the mixture for the lysine-deficient ration two molar equivalents of L-glutamic acid replaced the lysine.

† Composition given in reference 21.

intake. The amino acid mixture contained the L-amino acids in essentially the proportions found in whole egg(14) except that glycine was substituted for L-serine and L-glutamic acid for L-proline. In the lysine-deficient ration, L-glutamic acid was substituted in 2 molar proportions for L-lysine contained in the complete amino acid mixture. About 10 rats were in each of 7 groups. Group A was fed the complete ration for 14 days and then killed to determine the ratio of liver to body weights, a ratio used to estimate amount of liver not removed from Groups C and E. Groups B, C and F received the complete ration for 22 days; Groups D, E and G received the lysine-deficient ration for 22 days. Animals of Group C and E were partially hepatectomized on the 15th day, under ether anesthesia, by the method of Ralli and Dumm (15), a procedure by which an estimated 54.9 $\pm$ 3.67%‡ and 55.8 $\pm$ 3.0% respectively, of the liver were removed. On the same day groups B and D were subjected to sham operation which included anesthesia, exteriorization of the liver through the incision, replacement of liver and suturing. The rats were killed 8 days after operation when regeneration of liver can be essentially complete(6). Groups F and G served as non-operated controls. All animals were killed with chloroform. Whole livers, like the lobes which were removed by partial hepatectomy, were immediately weighed, partially dried at 60°C, then ground in glass mortar. The ground livers were analyzed for residual moisture by drying *in vacuo* at 90°C for 8 hours and for total lipids(16) and total N(17).

*Results.* In spite of lysine deficiency, rats in Group E were able to regenerate a major portion of the liver during 8 days, when their livers had attained 90.7 $\pm$ 3.4% of estimated fresh weight before hepatectomy; in Group C which consumed the complete ration the corresponding figure was 89.9 $\pm$ 2.1%.

As shown in Table II this increase in weight of liver, after partial hepatectomy, was accompanied by some changes in composition. In the lysine-deficient Group E, there was a significant increase in % moisture ($P = < 0.01$)§ and in lipids ($P = <0.01$) and a decrease in % N ($P = <0.01$). In Group C lipid content increased ($P = <0.01$) and the N content decreased ($P = <0.01$) while the change in % moisture was not significant. Considering the estimated amount of N remaining after partial hepatectomy and the final N content of livers, lysine-deficient rats deposited during recovery a mean of 51.7 $\pm$ 10.1 mg of N compared to 77.6 $\pm$ 16.1 mg for rats fed the complete ration. Calculated on a weight basis these values are 31.6 mg and 38.7 mg of nitrogen/100 g of final body weight respectively; the difference is not statistically significant. Formation of new liver tissue and deposition of N therein was accomplished by lysine-deficient rats in spite of the fact that during the preceding 2 weeks they had been in negative N balance, as evidenced by a loss of body weight, and that this condition continued during the period of regeneration.

Changes in composition of liver were partly due to surgical procedure. Comparison of sham operated groups B and D with the unoperated groups F and G respectively, revealed that the former had a significantly greater ($P = <0.01$) liver size with increased concentration of lipid ($P = <0.05$) than the rats fed either lysine-deficient or the complete ration ($P = <0.01$). Concentration of N was lower ($P = <0.05$) in livers of the sham

‡ Standard error of mean.

§ P = probability that the difference is due to chance.

TABLE II. Composition of Livers.

| Group | Lysine in diet, % | Operation* | No. of rats | Wt change, g | Liver | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Dry wt, g | % of body wt | Moisture, % | Lipid, % of dry wt | Nitrogen, % of dry wt |
| B | 1.38 | Sham | 9 | + .9 ± 2.0† | 2.4 ±.04 | 4.0 ±.07 | 70.5 ± .36 | 23.7 ±1.37 | 10.1 ±.10 |
| D | 0 | " | 10 | −26.2 ± 2.3 | 2.2 ±.12 | 4.2 ±.17 | 71.0 ±1.85 | 29.0 ±2.55 | 9.6 ±.41 |
| C | 1.38 | At P.H. | 11 | − 6.8 ± 3.6 | | | 70.1 ± .43 | 19.3 ± .61 | 11.0 ±.34 |
| | | When killed | 11 | − 2.6 ± 4.3 | 2.0 ±.15 | 3.4 ±.10 | 71.1 ±1.20 | 23.1 ± .92 | 9.7 ±.18 |
| E | 0 | At P.H. | 9 | −25.7 ± 1.3 | | | 70.5 ± .50 | 19.6 ± .73 | 10.4 ±.23 |
| | | When killed | 9 | −29.6 ± 1.6 | 1.5 ±.18 | 3.4 ±.18 | 72.7 ± .51 | 25.0 ± .83 | 9.4 ±.21 |
| F | 1.38 | Non-op. | 10 | +9.6 ± 2.8 | 2.1 ±.07 | 3.5 ±.11 | 71.2 ± .35 | 19.0 ± .41 | 10.8 ±.27 |
| G | 0 | " | 10 | −29.6 ± .90 | 1.6 ±.04 | 3.2 ±.06 | 70.9 ± .21 | 19.9 ± .66 | 10.2 ±.12 |

* Sham = sham operation; P.H. = partial hepatectomy; Non-op. = non-operated.
† Stand. error of mean.

operated animals (Group B) fed the complete diet than in livers of unoperated controls (Group F). In contrast, lysine deficiency had no effect on concentration of liver lipids, either after 14 days (compare Groups C and E) or 22 days (compare Groups F and G) of feeding. It did, however, cause a marked decrease ($P = <0.05$) in concentration of N in livers of non-operated control group but not in sham operated or partially hepatectomized groups.

Lysine deficiency caused a marked loss in body weight as would be expected from the decreased food consumption which was, for instance, a mean of 175 g/rat for the 22-day period in Group G, compared to a mean of 224 g in Group F. In lysine-deficient animals the loss of weight was not further accentuated by partial hepatectomy or sham operation, but in rats fed the complete diet the body weight was suppressed by these procedures.

The incisions made during hepatectomy or sham operation of normal animals healed more rapidly than those made into the lysine-deficient rats. The latter chewed on sutures and skin which often left raw areas. This was not observed in rats fed the complete ration. Impaired healing of wounds in protein-deficient rats has previously been reported(18).

The results of these experiments illustrate strikingly that, while the organism as a whole requires a dietary supply of all "essential" amino acids, deposition of nitrogenous compounds and, presumably, the net accumulation or synthesis of proteins can occur in individual tissues of rats fed a lysine-deficient ration. Under these conditions, the incomplete dietary supply of amino acids must have been locally balanced by amino acids which came from degradation of proteins in other, perhaps less vital tissues. Proliferating liver, in this instance, has what others(19) referred to as "priority in utilization of limiting amino acids." Mitchell(20) pointed to a somewhat analogous situation in which "depression in the output of endogenous nitrogen induced by ingestion of methionine may be due to the effect of this amino acid in arresting the raiding of protoplasmic tissues to secure cysteine for hair growth." The preferential synthesis of milk proteins in the mammary gland of animals which are in negative nitrogen balance, is another example of ability of the organism to control protein synthesis, to some extent at least, according to specific localized needs.

*Summary.* Rats maintained for 2 weeks on a lysine-deficient ration and then partially hepatectomized were able to regenerate their livers in spite of continued consumption of a lysine-deficient diet.

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## Comparative Uptake of Calcium-45 and Strontium-90 by Wild and Lordotic Guppies.* (23826)

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Investigations concerning metabolism of bone and mineralized tissues have been hampered by lack of suitable experimental animals. Recently, a naturally-occurring mutation in the guppy, *Lebistes reticulatus* (Peters) has been described which forms bone of greater density and higher calcium content (1). The mutation, a single recessive autosomal character, displays marked dorso ventral and lateral curvature of spinal column which has been called "hunchback"(2,3) or "lordosis"(4). Harrison(5) analyzed 3 animals and found that total body calcium is higher in the mutant fish(5). Rosenthal(1) demonstrated that calcium is increased in lordotic spines while muscle calcium concentration is not altered. The calcium:phosphorus ratios of lordotic bone is also greater since the phosphorus content of bones from both strains of fish is the same(1). These data strongly suggest a specific alteration of calcium metabolism is osseous tissues from lordotic animals.

A series of studies with radioactive calcium-45 and strontium-90 was undertaken in order to determine if the metabolic defect leading to an accumulation of calcium in osseous tissues of the lordotic guppy was due to alterations in the rate of incorporation and/or turnover of mineral elements.

*Materials and methods.* Wild type guppies were obtained from commercial sources and lordotic guppies were raised in this laboratory, (6). The general experimental design, isotope technics and analytical methods have been previously published in detail(1,7-9). In the present comparative studies with wild

* Neutralizing stones were analyzed for calcium and strontium by Crippen and Ehrlich Laboratories, Baltimore, Md. We are grateful for the spectrographic strontium analysis of bone ash performed through the courtesy of Dr. Karl K. Turekian, Yale University. This investigation was performed under contract with the U. S. Atomic Energy Comm.