INFLUENCE OF STARVATION ON INCORPORATION OF URACIL-2-C^{14} IN LIVER AND HEPATOMA RNA IN THE RAT*

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The data to be presented indicate that starvation results in active in vivo utilization of uracil for liver RNA synthesis in the rat. This experimental state, superficially at least, differs from those in which this pathway of RNA biosynthesis has been demonstrated previously in rat liver.

Materials and Methods

All experiments were performed on adult male rats obtained from Barkbridge Farms, N.J., maintained on a stock diet of Purine Fox Chow. Studies involving the transplanted hepatoma were made 10-14 days after implantation of a saline suspension of hepatoma cells subcutaneously in the groin of A x C rats, the strain in which this neoplasm was produced originally by feeding diacetylaminofluorene (by H. Morris). The other studies were made on Wistar descendants.

Animals in all experimental groups received 20 mg. of uracil-2-C^{14} (500 μc/millimole)** in 10 ml of 0.85 per cent NaCl solution intraperitoneally 18 hours prior to sacrifice by cervical fracture and decapitation; previous studies showed maximum incorporation in liver nucleic acids at about this time(Rutman et al. 1954). After exsanguination, the livers were perfused in situ with cold 0.25M sucrose solution and (also subcutaneous tumors) were excised and placed on cracked ice.

In the starvation experiments, all food was withheld for four days prior to sacrifice (water ad libitum). In those involving testosterone,

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this was injected as the propionate, 10 mg. every other day for twelve days prior to injection of the uracil.

The livers and tumors were blotted dry, weighed, minced with scissors, and homogenized at 0°C. in 0.25M sucrose (1:10) in a Potter-Elvehjem homogenizer. Analyses were performed in duplicate on individual livers and tumors. RNA was isolated in a conventional manner (Herbert et al. 1957) and was analyzed by the orcinol reaction (Mejbaum 1939) and phosphorus determination (Allen 1940; Schneider 1945). Samples were plated by evaporation and counted in a gas-flow counter to ± 5 per cent accuracy.

Results and Discussion

The pertinent results are presented in the table.

Specific Activity of RNA after Intraperitoneal Injection of 20 mg. Uracil-2-C^{14} (3.3×10^6 counts/min./mg.)

No. of Rats	Experiment	Av. Weight Change (grams)	Specific Activity* (counts/min/mg RNA)	
			Liver	Tumor
2 A x C	Stock Diet	+7	182 ± 26	
3 A x C	Starvation 4 days	-32	875 ± 136	
3 A x C	Transplanted Hepatoma Stock Diet	+6**	698 ± 120	1354 ± 268
3 A x C	Transplanted Hepatoma Starvation 4 days	-35**	645 ± 116	2883 ± 426
4 Wistar	Stock Diet	+9	115 ± 22	
4 Wistar	Starvation 4 days	-35	1084 ± 284	
4 Wistar	Testosterone Stock Diet	+12	690 ± 98	
4 Wistar	Testosterone Starvation 4 days	-30	1704 ± 388	

*All values are means, with mean deviations.
** Carcass (rat minus tumor) weight.

There was no difference in uracil incorporation in the liver RNA of untreated Wistar and A x C rats. In these animals, starvation (4 days) resulted in increased incorporation, to levels approximating those observed in regenerating liver (Cantarow et al. 1958) and hepatoma. Similarly, the active incorporation induced by testosterone was increased further by

starvation. In rather sharp contrast to this consistent effect in these experimental groups, no augmentation of the initially active incorporation occurred in the livers of rats bearing the transplanted hepatoma. There was an apparent change in this direction, however, in the tumor RNA in the starved animals.

The increased rate of incorporation of uracil in the liver RNA of rats bearing the transplanted hepatoma is in qualitative agreement with the findings of Heidelberger et al.(1957) in rats bearing the Flexner-Jobling carcinoma. There are several reports of an increase in liver RNA in tumor-bearing animals (Leslie 1955). However, the rate of incorporation of P^{32} in the liver RNA has not been found to differ significantly from that in normal animals (Payne et al. 1952; Tyner et al. 1953). A similar situation exists in pregnant rats, in which the total liver RNA is increased, with no increase in the specific activity of RNA-P after administration of P^{32} (Campbell et al. 1953), whereas we have observed active incorporation of uracil in the liver RNA (unpublished observations).

Although there is a rather extensive literature on nutritional and hormonal influences on RNA (Leslie 1955), there are very few reported observations that are relevant to those presented here. The total liver RNA and also its concentration per unit fresh weight decreases on a protein-deficient diet (Jacob et al. 1951; Thomson et al. 1953). On a protein-free, high-caloric (1750 kg. cal./sq. meter) diet, the decrease in RNA was associated with an increased rate of incorporation of P^{32} (Munro et al. 1953). Lowering the energy content of the diet (protein-free) to 815 kg. cal./sq. meter resulted in lowering of the relative specific activity of the liver RNA-P, which, however, still exceeded that in rats on a diet adequate in protein and calories. Although no similar studies were made under fasting conditions, these observations may perhaps be related to the increased rate of incorporation of uracil in liver RNA in starved animals.

All rat tissues in which active in vivo incorporation of uracil into RNA had been demonstrated previously were (a) neoplastic or "preneoplastic"

or (b) rapidly growing (regenerating liver; intestinal mucosa)(Rutman et al. 1954; Heidelberger et al. 1957), or (c) were exposed to the action of protein anabolic agents (growth hormone, testosterone)(Cantarow et al. 1958) or (d) showed evidence of increased protein synthesis (livers of pregnant and of tumor-bearing rats). In view of the observations reported here, a common denominator of stimulation of growth is not applicable, nor, apparently, is that of an increased rate of turnover of RNA, at least as reflected in the rate of incorporation of P^{32} (increased in tumors, regenerating liver, possibly also starvation liver; unchanged in livers of pregnant and of tumor-bearing rats). However, in this connection, more precise information is required concerning incorporation of P and of uracil in the various cell fractions of RNA under these experimental conditions.

In view of current interest in in the tumoristatic activity of 5-fluorouracil, the metabolism of which parallels that of uracil (Chaudhuri et al. 1958), the influence of nutritional and endocrine factors on this metabolic pathway may have certain practical implications.

REFERENCES

Allen, R.J.L., Biochem. J., 34:858, 1940
Campbell, R.M., Innes, I.R., and Kosterlitz, H.W., J. Endocrinol. 9:52,1953
Cantarow,A., Williams,T.L., Melnick,I., and Paschkis,K.E., Cancer Research 18:818, 1958
Chaudhuri,N.K., Montag,B.J., and Heidelberger,C., Cancer Research 18:318,1958
Heidelberger,C., Leibman,K.C., Harbers,E., and Bhargava,P.M., Cancer Research 17:399, 1957
Herbert,E., Potter,V.R., and Hecht,L.I., J. Biol. Chem., 225:659, 1957
Jacob,M., Mandel,L., and Mandel,P., Experientia 7:269, 1951
Leslie,I., in The Nucleic Acids (E.Chargaff and J.N.Davidson, Ed.), New York, Academic Press, Inc., vol.II, pp. 24, 32, 1955
Mejbaum,W.Z., Z. physiol. Chem., 258:117, 1939
Munro,H.N., Naismith,D.J., and Wikramanayake,T.W., Biochem.J.,54:198,1953
Payne,A.H., Kelly,L.S., and White,M.R., Cancer Research 12:65, 1952
Rutman,R.J., Cantarow,A., and Paschkis,K.E., Cancer Research 14:119,1954
Schneider,W.C., J. Biol. Chem., 161:293, 1945
Thomson,R.Y., Heagy,F.C., Hutchison,W.C., and Davidson,J.N., Biochem.J. 53:460, 1953
Tyner,E.P., Heidelberger,C., and LePage,G.A., Cancer Research 13:186,1953

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