FURTHER STUDIES OF ELECTRON TRANSPORT COMPONENTS IN A SERIES OF MORRIS HEPATOMA-BEARING RATS

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Summary.—Microsomal electron transport components (NADPH oxidase, NADPH-ferricyanide reductase, cytochromes P-450 and b_5) have been studied in Buffalo-strain rat liver and in a series of Morris hepatomata (9618A-2, 7800, 7795 and 7787). Normal liver values differed significantly from those measured in livers of tumour-bearing animals. In all hepatomata *per se*, very low levels were found.

KATO and associates (1963, 1968) have reported that in rats bearing carcinosarcoma 256 the oxidation rate of drugs by liver microsomes was significantly lower than normal. Sugimura *et al.* (1966) have found microsomal enzyme activity in 4 Morris hepatomata at equivalent or somewhat lower levels than that found in normal or regenerating liver. Markedly different levels of activities of microsomal NADPH oxidase, ferricyanide reductase, P-450, cytochrome b_5 and benzpyrene hydroxylase were also found when the normal liver was compared with Morris hepatomata 7777-bearing rat liver (Brown *et al.*, 1971). The present report is a continuation of this study of electron transport components in control Buffalo-strain rat liver and in Morris hepatomata 9618A-2, 22nd generation (fast-growing tumour); 7800, 54th generation (medium-growing tumour); 7795, 45th generation (medium-growing tumour); and 7787, 16th generation (slow-growing tumour) (Morris and Wagner, 1968).

MATERIALS AND METHODS

Female Buffalo-strain rats (3–8 months old, average weight 220 g) with bilateral tumours in the hind leg and non-tumour-bearing control animals from the same stock

were used. The tumours had been carried by serial transplantation. Animals were shipped by air and upon arrival were kept separately in a temperature controlled room. The rats were sacrificed in several batches. The first batch was used approximately 8 weeks after arrival and 60 days after inoculation. Control and tumour-bearing animals were used simultaneously. The animals were stunned and decapitated and the liver (average wet wt 12 g) was removed and homogenized immediately in 3 volumes of 1.15% KCl solution in a Waring blender. The slurry was further homogenized in a power-driven Teflon-pestle glass homogenizer. The homogenate was centrifuged $9000 \times g$ for 20 min. Pellets were rejected and the supernatant was further centrifuged in 10 ml tubes at $105,000 \times g$ for 90 min. Pellets from the last centrifugation were used immediately or stored at -30°C . Through all manipulation a temperature of 2.4°C was maintained. For use, a pellet was resuspended in 0.1 mol/l phosphate buffer, pH 7.45. Enzymic activities were measured spectrophotometrically using a Cary 15 spectrophotometer. NADPH oxidase was assayed by the method of Gillette *et al.* (1957); ferricyanide reductase was assayed following the method of Williams and Kamin (1962). Protoheme P-450 (reduced, carbon-monoxide-bound complex) and cytochrome b_5 were assayed by difference spectrum according to the method of Omura and Sato (1964) and Fouts (personal communication), respectively.

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TABLE I.—*Enzymes of Microsomal Electron Transport System and Cytochrome P-450 and b₅ in Buffalo Rat Liver and Hepatoma Tissue*

Enzyme	Hepatoma type	Age of tumours studied (days)	Liver (normal)	Liver (hepatoma bearing)	Change (%)	Significance (P <)	Hepatoma
NADPH oxidase (10 ⁻⁹ mole/mg protein/min)	9618A-2	58-75	11.6±1.6	7.5±0.6	-35	0.001	2.5±0.6
	22nd gen.						
	7800,	60-78	12.2±2.0	7.0±2.2	-43	0.001	2.1±1.2
	54th gen.						
	7795,	75-90	10.7±0.3	5.8±1.3	-46	0.002	1.1±0.2
NADPH-ferri- cyanide reductase (10 ⁻⁹ mole/mg protein/min)	45th gen.						
	7787,	118-150	13.1±3.7	10.0±2.4	-24	0.10	2.1±0.8
	16th gen.						
	9618A-2		25.5±6.5	12.2±6.6	-52	0.003	4.4±1.4
Cytochrome P-450 (10 ⁻⁹ mole/mg protein)	7800		17.3±6.9	8.1±1.0	-53	0.005	4.5±2.6
	7795		19.2±3.1	10.3±3.2	-46	0.05	7.8±0.3
	7787		22.1±11.1	13.8±4.4	-38	0.02	2.3±0.3
Cytochrome b ₅ (ΔOD ₄₂₅₋₄₁₀ /mg protein)	9618A-2		1.0±0.1	0.8±0.2	-20	0.01	—
	7800		1.1±0.1	0.8±0.1	-27	0.001	—
	7795		1.2±0.1	0.9±0.1	-25	0.03	—
	7787		1.4±0.3	1.2±0.3	-14	0.20	—
Cytochrome b ₅ (ΔOD ₄₂₅₋₄₁₀ /mg protein)	9618A-2		0.11±0.04	0.08±0.04	-27	0.02	—
	7800		0.11±0.02	0.09±0.02	-18	0.02	—
	7795		0.10±0.01	0.09±0.01	-10	0.05	—
	7787		0.11±0.01	0.08±0.01	-27	0.001	—

Each value represents a mean of two extreme points obtained from 5 rats.

RESULTS AND CONCLUSIONS

NADPH oxidase, ferricyanide reductase, protoheme P-450 and cytochrome b₅ were present at lower than normal activity levels in the liver of tumour-bearing animals. Very low levels of NADPH oxidase and ferricyanide reductase were present in all the tumours examined. P-450 and cytochrome b₅ were not measurable in any of the tumours (Table I).

Present observations indicate that the growth rate of the Morris hepatomata examined (fast, medium and slow-growing types were used) does not correlate with activity levels of microsomal electron transport components.

These results, similar to those of Kato *et al.* (1968) for Walker carcinosarcoma 256 and those of Brown *et al.* (1971) for Morris hepatoma 7777, do indicate a lowered activity level of liver microsomal haemoprotein and a decreased activity of enzymes of the NADPH-linked electron transport system.

The interpretation of the lower levels of certain elements of the NADPH-

electron transport system in livers and hepatomata of tumour bearing rats, as a causal relationship in the development of the tumour or as a secondary consequence, cannot at this point be distinguished.

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REFERENCES

- BROWN, H. D., CHATTOPADHYAY, S. K., PENNINGTON, S. N., SPRATT, J. S. & MORRIS, H. P. (1971) Mixed-function Oxidation in Tumours. *Br. J. Cancer*, **25**, 135.
- GILLETTE, J. R., BRODIE, B. B. & LADU, B. N. (1957) The Oxidation of Drugs by Liver Microsomes: on the Role of TPNH and Oxygen. *J. Pharmac. exp. Ther.*, **119**, 532.
- KATO, R., FRONTINO, G. & VASSANELLI, P. (1963) Decreased Activities of Liver Microsomal Drug Metabolizing Enzymes in the Rats Bearing Walker Carcinosarcoma. *Experientia*, **19**, 31.
- KATO, R., TAKANAKA, A. & TAKAHASHI, A. (1968) Activities of NADPH-linked Electron Transport System in Tumour-bearing Rats. *Gann*, **59**, 83.
- MORRIS, H. P. & WAGNER, B. P. (1968) Induction and Transplantation of Rat Hepatomas with Different Growth Rate (Including "Minimal Deviation" Hepatomas). In *Methods in Cancer Research*, Vol. 4. Ed. Harris Busch. New York: Academic Press. p. 125.

- OMURA, T. & SATO, R. (1964) The Carbon Monoxide Binding Pigment of Liver Microsomes. I. Evidence for its Hemoprotein Nature. *J. biol. Chem.*, **239**, 2370.
- SUGIMURA, T., MATSUSHIMA, T., KAWACHI, T., HIRATA, Y. & KAWABE, S. (1966) Molecular Species of Aldolases and Hexokinases in Experimental Hepatomas. *Gann*, **1**, 143.
- WILLIAMS, C. H. & KAMIN, H. (1962) Microsomal Triphosphopyridine Nucleotide-cytochrome c Reductase of Liver. *J. biol. Chem.*, **237**, 587.