10. J. H. FLEISHER, H. O. MICHEL, L. YATES and S. C. HARRISON, *J. Pharmac.* **129**, 31 (1960).
11. H. OETTEL, *Dtsch. Ärztbl.* **64**, 2720 (1967).
12. H. PRINZ, *Dtsch. Ärztbl.* **64**, 1845 (1967).
13. J. BAJGAR and J. PATOČKA, *Sborník věd. prací VLVDÚ* (Hradec Králové) **44**, 3 (1969).
14. G. L. ELLMAN, D. K. COURTNEY, V. ANDERS, JR. and R. M. FEATHERSTONE, *Biochem. Pharmac.* **7**, 88 (1961).
15. M. HRAŠKA and J. TULACH, Contribution to automatization of evaluation of experimental data in experimental medicine. Programmes for MINSK 22 computer. VLVDÚ Hradec Králové (1966).
16. J. BAJGAR and R. URBAN, *Suppl. Sborníku věd. prací LF KU Hradec Králové* **13**, 219 (1970).
17. J. BAJGAR, *Sborník věd. prací VLVDU (Hradec Králové)* **44**, 57 (1969).
18. J. BAJGAR and A. JAKL, *Voj. zdravotn. Listy* **39**, 253 (1970).
19. C. A. DE CANDOLE, W. W. DOUGLAS, L. C. EVANS, R. HOLES, K. E. SPENCER, R. W. TORRANCE and K. M. WILSON, *Br. J. Pharmac.* **8**, 466 (1953).
20. J. HERINK, J. BAJGAR and J. PATOČKA, *Voj. zdravotn. Listy* **39**, 191 (1970).

Biochemical Pharmacology, Vol. 20, pp. 3233–3236. Pergamon Press, 1971. Printed in Great Britain

Effect of diethylnitrosamine on the respiratory and enzymic response of rat liver to corticosterone

(*Received* 16 *January* 1971; *accepted* 13 *May* 1971)

CHANGES in the respiratory and enzymic activity of rat liver preparations induced *in vitro* by incubation with corticosterone have been shown[1] to be associated with the saturation of two types of hormone receptor sites.[2] Hormonal action on tissue respiration has been associated with increased activity of enzymes of the tricarboxylic acid cycle and an increased transaminase activity of the tissue.[3] The regulatory response of gluconeogenic enzyme activity and glycogen level in rat liver to glucocorticoid injection has been described by Webber *et al.*[4] and it has been shown that this hormonal response is blocked by inhibitors of protein synthesis. The steroid response was found to be limited in slow growing hepatomas and absent in fast growing hepatomas.[5]

In the present work we have studied the change in corticosterone response of rat liver preparations taken from rats fed diethylnitrosamine[6] with a normal diet (Oxoid 86), in order to investigate effects of carcinogens leading to a loss of hormone response.

A group of 20 male rats (8 week old) were fed diethylnitrosamine (50 mg/l.) in the drinking water over a period of 6 months. A group of 20 litter mates were kept over the same period as control animals. At the start of the experiment six male rats were killed by a blow on the head and the livers exsanguinated by infusion of 0·9% heparinized saline via the hepatic portal vein. Animals for each experiment were killed at mid-morning,[7] the livers were excised, pooled and minced using a stainless steel press through 1·2-mm dia. holes. The oxygen consumption, succinate dehydrogenase activity and tyrosine transaminase activity were measured in this liver preparation using procedures described previously by Dalton and Snart[1] in the presence and absence of 10^{-9}M and 10^{-7}M corticosterone. These studies were repeated at the 3 and 6 month stage of treatment using six treated and control animals at each stage. The livers taken from animals at the 6 month stage of treatment with diethylnitrosamine showed tumor development as evidenced by an observed increase in size and apparent surface hepatomas with some necrosis. However, no detailed examination of the tissue was undertaken. All the tissue was used in preparation of the experimental sample. A 100,000 *g* supernatant protein was obtained from a 50 per cent homogenate of livers taken from 6 month treated and control animals. Each supernatant fraction was incubated with low specific activity ^{3}H corticosterone for 1 hr at 20°, after which 1 ml of each sample was applied to a 2 × 50 cm Sephadex G50 column and the corticosterone binding capacity of the liver protein measured in terms of the amount of radioactivity eluting with the protein peak.

The enzymic response of the control liver, Fig. 1, confirmed our previous observation that the succinate hydrogenase activity is maximally stimulated by 10^{-9}M corticosterone, whereas the tyrosine transaminase activity was maximally stimulated by 10^{-7}M corticosterone. The level of succinate

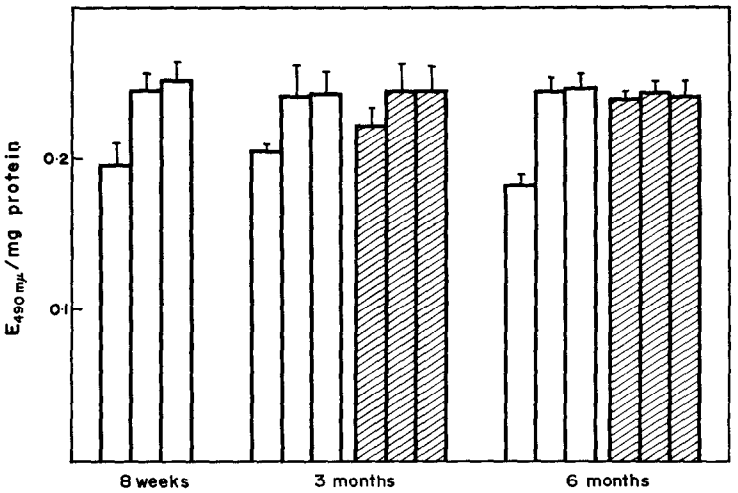

FIG. 1. Succinate dehydrogenase activity (E_{490}/mg protein) measured in rat liver homogenates. The histograms represent the mean of four determinations with the S.E.M. and are arranged in order to show the enzyme activity in the absence and presence of 10^{-9} and 10^{-7} M corticosterone. The open histograms represent values obtained using control animals and the cross hatched histograms correspond to values obtained using diethylnitrosamine treated animals, at the time period indicated.

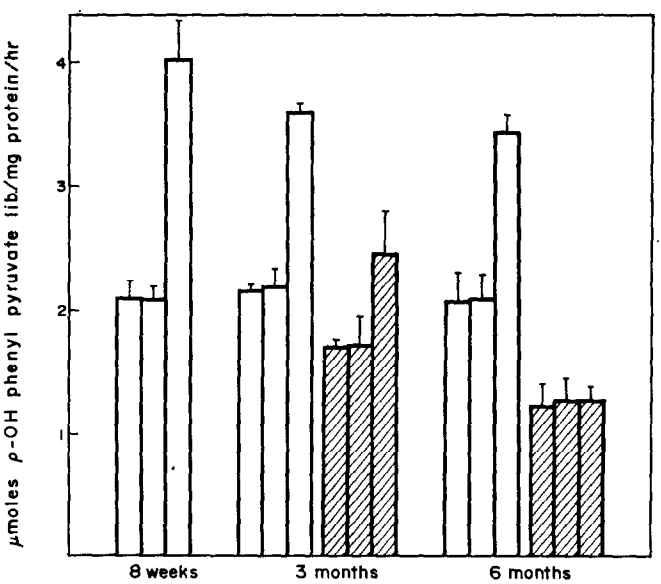

FIG. 2. Tyrosine transaminase activity (μmoles *p*-hydroxyphenyl pyruvate liberated 1 hr/mg protein) measured in rat liver homogenates in the absence and presence of 10^{-9} and 10^{-7}M corticosterone. The results are expressed in a manner corresponding to that described in the legend to Fig. 1.

TABLE 1. OXYGEN CONSUMPTION (μl O_2 consumed/mg dry weight/hr) IN RAT LIVER PREPARATIONS OBTAINED FROM CONTROL AND DIETHYLNITROSAMINE TREATED ANIMALS AT THE 3 AND 6-month STAGE OF TREATMENT IN THE PRESENCE AND ABSENCE OF 10^{-9} AND 10^{-7}M CORTICOSTERONE

Time period (hr)		3 month		6 month	
		untreated	DENA treated	untreated	DENA treated
No hormone	1st	1·23 ± 0·07	1·48 ± 0·09	1·24 ± 0·04	1·54 ± 0·06
	2nd	1·29 ± 0·29	1·42 ± 0·15	1·21 ± 0·05	1·54 ± 0·07
	3rd	1·31 ± 0·31	1·40 ± 0·12	1·18 ± 0·05	1·53 ± 0·06
	4th	1·18 ± 0·17	1·36 ± 0·03	1·17 ± 0·05	1·53 ± 0·06
10^{-9}M corticosterone	1st	1·28 ± 0·14	1·46 ± 0·07	1·30 ± 0·13	1·51 ± 0·05
	2nd	1·40 ± 0·11	1·54 ± 0·07	1·33 ± 0·15	1·54 ± 0·10
	3rd	1·49 ± 0·09	1·56 ± 0·05	1·39 ± 0·12	1·56 ± 0·09
	4th	1·46 ± 0·12	1·55 ± 0·04	1·38 ± 0·12	1·50 ± 0·07
10^{-7}M corticosterone	1st	1·46 ± 0·19	1·47 ± 0·05	1·33 ± 0·17	1·57 ± 0·06
	2nd	1·62 ± 0·18	1·57 ± 0·06	1·54 ± 0·09	1·53 ± 0·09
	3rd	1·73 ± 0·14	1·64 ± 0·07	1·70 ± 0·08	1·54 ± 0·06
	4th	1·80 ± 0·15	1·73 ± 0·06	1·75 ± 0·13	1·49 ± 0·08

The results represent the mean ± S.D. of ten values.

TABLE 2. BINDING CAPACITY (moles/mg wet weight) OF RAT LIVERS TAKEN FROM CONTROL ANIMAL AND 6-month TREATED ANIMALS

	Binding capacity (moles/mg wet wt.)
Control animals	$5{\cdot}6 \times 10^{-12}$
Diethyl nitrosamine treated animals	$2{\cdot}5 \times 10^{-12}$

This single determination on a pooled sample was made by incubation of 1 ml of a 200,000 *g* supernatant protein fraction obtained from a 50 per cent homogenate of each pooled liver sample, with 1 μc [^{3}H]corticosterone (10^{-9} moles) at 20°, followed by separation on a 2 × 50 cm Sephadex G50 column. The activity was counted using Bray's scintillation fluid with an automatic quenching correction.

dehydrogenase activity in the tissue shows an increase following diethylnitrosamine (DENA) treatment, that after 6 months treatment reaches a value that compares with the level of maximum activity obtained following hormonal stimulation. The loss of hormonal stimulation may therefore result from the fact that a maximum enzyme activity has already been stimulated by the carcinogen. Tyrosine transaminase on the other hand Fig. 2, shows a decreased activity in parallel with the observation of Webber.[5] The decrease in hormone response is significant after 3 months treatment and complete after 6 months treatment. Changes in oxygen consumption (Table 1) are less obvious than the changes in enzyme activities but the results are in keeping with those expected from a loss of hormone responsiveness, following diethylnitrosamine treatment. Although the characterization of the hormone binding in the tissue has not been carried out conclusively (Table 2), the binding capacity was found to be reduced following diethylnitrosamine treatment reaching a value that may correspond to residual transcortin binding in the tissue.

It is tentatively suggested that an effect of the carcinogen may be to cause a deletion of hormone receptor proteins with a subsequent loss of hormonal activity. If these receptor proteins are considered part of the protein synthesis control in the target tissue, then a role of carcinogens may be to cause specific protein deletion as suggested by Miller and Miller.[8]

Department of Zoology,
The University,
Sheffield S10 2TN

T. DALTON*
R. S. SNART

REFERENCES

1. T. DALTON and R. S. SNART, *J. Endocrin.* **47**, 159 (1970).
2. R. S. SNART, N. N. SANYAL and M. K. AGARWAL, *J. Endocrin.* **47**, 149 (1970).
3. R. S. SNART, *Hormones* **1**, 233 (1970).
4. G. WEBBER, R. L. SINGHAL, W. B. STAMM, E. A. FISHER and M. A. MENTENDINCK, *Adv. Enzyme Reg.* **2**, 1 (1964).
5. G. WEBBER, *Adv. Enzyme Reg.* **1**, 321 (1963).
6. P. N. MAGEE and J. M. BARNES, *Br. J. Cancer* **10**, 114 (1956).
7. V. R. POTTER, R. A. GEBERT and H. C. PITOT, *Adv. Enzyme Reg.* **4**, 247 (1966).
8. E. C. MILLER and J. A. MILLER, *Cancer Res.* **12**, 547 (1952).

* Present address: Department of Zoology, Westfield College, London.

Biochemical Pharmacology, Vol. 20, pp. 3236–3238. Pergamon Press, 1971. Printed in Great Britain

Acceleration by free carbamate of the spontaneous reactivation of carbamylated acetylcholinesterase

(*Received* 10 *March* 1971; *accepted* 13 *May* 1971)

SEVERAL workers have shown that inhibition of acetylcholinesterase (AChE; EC 3.1.1.7) by carbamates is adequately described by the mechanism[1–3]

$$E + I \xrightarrow{k_i} EI' \xrightarrow{k_r} E + \text{products} \qquad (1)$$

where E is the enzyme, I a carbamate and EI' a carbamyl enzyme. *In vitro* the velocity declines to a steady state at which rates of inhibition and spontaneous reactivation are equal. The forward bimolecular rate is first order because the concentration of inhibitor is greatly in excess of that of enzyme. Previous workers have estimated inhibition rates by a method which involves discarding some of the data of progress curves, viz. those beyond the range in which a semilogarithmic plot of velocity against time is sensibly linear. Reactivation rates have usually been estimated by greatly diluting enzyme–inhibitor mixtures and observing the rate of increase of velocity. Reiner and Simeon-Rudolf[2] have also estimated them by multiplying the inhibition rate constant by the "equilibrium constant" obtained by assuming that the steady state represents a true equilibrium. A different method, which uses all the data from progress curves, and which has not previously been used in studies of AChE, is based on the assumption that the approach to a steady state is kinetically equivalent to approach to a true equilibrium, a mathematical treatment for which has been presented.[4] Since both forward and reverse reactions are first order the approach to equilibrium is also first order, and is related to the initial velocity v_o, the equilibrium velocity v_e and the intermediate velocities v at times t thus:

$$k = \frac{2{\cdot}303}{t} \log \left(\frac{v_o - v_e}{v - v_e}\right). \qquad (2)$$

This rate is also the sum of the forward and reverse rates:

$$k = k_i(I) + k_r. \qquad (3)$$