Because of the general use by Bracha and O'Brien of method B in the synthesis of their compounds, there must now arise doubts as to the validity of their results and of their conclusions as to the factors affecting the rates of inhibition and on the relationship between the rates of inhibition and the toxicities of their compounds.

Chemical Defence Establishment,
Porton Down,
Salisbury,
Wiltshire, England

MICHAEL F. GAZZARD
GORDON L. SAINSBURY
DENNIS W. SWANSTON
DAVID SELLERS
PETER WATTS*

* Author to whom communications should be addressed.

REFERENCES

1. P. BRACHA and R. D. O'BRIEN, *Biochemistry* **7**, 1545 (1968).
2. P. BRACHA and R. D. O'BRIEN, *Biochemistry* **7**, 1555 (1968).
3. P. BRACHA and R. D. O'BRIEN, *Biochemistry* **9**, 741 (1970).
4. W. N. ALDRIDGE and A. N. DAVISON, *Biochem. J.* **51**, 62 (1952).
5. A. D. F. TOY, *J. Am. chem. Soc.* **70**, 3884 (1948).

Biochemical Pharmacology, Vol. 23, pp. 752–754. Pergamon Press, 1974. Printed in Great Britain.

Effect of certain metabolic regulators on the activity of lysosomal enzymes in regenerating rat liver

(*Received* 11 *June* 1973; *accepted* 13 *August* 1973)

AFTER partial hepatectomy livers undergo numerous metabolic changes in order to maintain homeostasis, which is known to be associated with the process of rapid cell multiplication and DNA formation, along with some definite changes in RNA and protein synthesis.[1–3] Recently it has been noted that during liver regeneration, concentration of DNA in the liver is increased, while that of NAD is decreased and it is suggested that NAD inhibits the process of mitosis during liver regeneration.[3] Information is also available regarding the regulation of synthesis of several enzymes such as DNase, alkaline phosphatase and deoxythymidinekinase in the regenerating livers under the influence of certain metabolic regulators.[4–6] Administration of hydrocortisone is known to induce ornithine decarboxylase in resting liver but it has no effect on this enzyme in regenerating liver, although it inhibits the process of DNA synthesis during the process of liver regeneration.[7] This indicates that the mechanism of regulation of enzyme synthesis in regenerating liver is different from that of normal liver. In this communication, investigations on the effects of certain metabolic regulators such as ACTH, c-AMP and glucagon on the activities of several lysosomal enzymes during the process of liver regeneration have been presented.

Male albino rats weighing between 90–100 g were used as experimental animals. One third of the liver was removed from the experimental group as described by Ferris and Clark[3] and rats of all groups were then maintained on 2% glucose solution. The different groups of animals are mentioned in Table 1. ACTH (0·1 IU/100 g), glucagon (100 μg/100 g) and c-AMP (200 μg/100 g) were injected to the respective groups of animals 3 hr before sacrifice. The rats were sacrificed 18 hr after partial hepatectomy to collect the regenerated part of liver for biochemical assay. Lysosome rich mitochondrial fractions have been used as enzyme sources and these have been prepared according to the method as described earlier.[8] For the disruption of lysosomes, Triton X-100 (0·2 per cent, v/v) was used. The activity obtained after treatment with Triton X-100 is designated as total activity. Assays for β-glucuronidase, acid phosphatase and alkaline phosphatase in the lysosomal fractions were carried out according to Michell *et al.*[9] Protein contents of the enzymes were determined by the method of Lowry *et al.*[10]

It is noted from Table 1 that the activities of acid and alkaline phosphatase are increased in regenerating liver as compared to the normal; while studying β-glucuronidase it is noted that there is an increase in the total activity of this enzyme in the regenerated system. Administration of c-AMP to the regenerating group of rats markedly inhibits the activities of acid and alkaline phosphatase but ACTH administration

to the experimental animals does not show any change in respect of these two enzymes. Similar observations on the effect of ACTH and c-AMP were also noted by other workers in respect of lysosomal acid phosphatase in the adrenals of rats.[11] These two metabolic regulators act in a different way in respect of lysosomal β-glucuronidase. Administration of ACTH depresses the activity of β-glucuronidase as compared to the regenerating groups of animals but c-AMP can, however, stimulate only the total activity of lysosomal β-glucuronidase. Treatment with glucagon, however, stimulates the activity of lysosomal alkaline phosphatase but marked inhibition is noted in the cases of acid phosphatase and β-glucuronidase. It is suggested that during liver regeneration, synthesis of the above mentioned enzymes might be stimulated and this could also be substantiated from the observations when lysosomes are treated with Triton X-100. It is concluded that c-AMP regulates these enzymes in regenerating liver by stabilizing the lysosomal membrane primarily, although its actions are not defined in respect of all the three enzymes as studied here.

TABLE 1. EFFECT OF CERTAIN METABOLIC REGULATORS ON THE ACTIVITY OF LYSOSOMAL ENZYMES IN REGENERATING RAT LIVER

System	Acid phosphatase (μg of *p*-nitrophenol liberated/min per mg of protein)	Alkaline phsophatase (μg of *p*-nitrophenol (liberated/min per mg of protein)	β-glucuronidase (μg of *p*-nitrophenol liberated/min per mg of protein)
1. Normal	3·64 ± 0·53 (6·64 ± 1·47)	1·18 ± 0·46 (1·61 ± 0·54)	25·60 ± 2·34 (48·38 ± 6·84)
2. Sham-operated	3·72 ± 0·87 (6·52 ± 1·24)	1·21 ± 0·64 (1·58 ± 0·96)	25·75 ± 1·56 (50·32 ± 5·71)
3. Regenerating	5·41 ± 0·35* (8·06 ± 2·00)	3·69 ± 0·49§ (6·37 ± 1·61)§	25·00 ± 1·18 (57·40 ± 2·17)†
4. Regenerating + ACTH	5·62 ± 0·60 (7·79 ± 1·30)	3·68 ± 0·52 (6·72 ± 1·30)	21·60 ± 0·67† (53·28 ± 7·02)†
5. Regenerating + c-AMP	3·79 ± 0·42§ (6·55 ± 0·64)	2·89 ± 0·60† (5·21 ± 0·69)	25·07 ± 2·35 (66·36 ± 2·44)‡
6. Regenerating + glucagon	4·96 ± 0·24† (6·75 ± 1·36)	4·54 ± 0·28* (7·13 ± 0·55)	18·24 ± 0·72* (45·68 ± 5·04)

The data inside the parentheses denote the total activity of the enzyme. Groups 2 and 3 have been compared with group 1, and groups 4, 5 and 6 have been compared with group 3. Mean values significantly different from the control group of animal:

* $P < 0.01$;

† $P < 0.05$; and

‡ $P < 0.02$.

§ Mean values different from the control group of animal very much significantly ($t > 5.96$ for 6° freedom).

Each result is expressed as mean ± S.D. of four experiments, each on a different animal.

Acknowledgement—This research has been financed in part by grant No. FG-IN-467, A7-HN-26 made by the U.S. Department of Agriculture under Public Law 480.

Department of Biochemistry,
University College of Science,
35, *Ballygunge Circular Road,*
Calcutta—700019, *India*

JABA CHATTERJEE
DIPALI RUDRA PAL
PRABIR K. MAJUMDER
GORA C. CHATTERJEE

REFERENCES

1. J. SHORT, R. F. BROWN, A. HUSAKORA, J. R. GILBERTRON, R. ZENAL and I. LIEBERMAN, *J. biol. Chem.* **247**, 1757 (1972).
2. M. G. ORD and L. A. STOCKEN, *Biochem. J.* **129**, 175 (1972).

3. G. M. FERRIS and J. B. CLARK, *Biochem. J.* **128,** 869 (1972).
4. V. ALLFREY and A. E. MIRSKY, *J. gen. Physiol.* **36,** 227 (1952).
5. J. M. PEKARTHY, J. SHORT, A. I. LANSING and I. LIEBERMAN, *J. biol. Chem.* **247,** 1767 (1972).
6. N. AKAMATSU, T. KAMIYA, H. R. MAEDA, N. ENDO, N. FUKUI and Y. MIURA, *J. Biochem.* **69,** 1091 (1971).
7. E. CAVIA and T. E. WEBB, *Biochem. biophys. Acta* **262,** 564 (1972).
8. P. BODENOCH-JONES and H. BAUM, *Nature, New Biol.* **242,** 123 (1973).
9. P. H. MICHELL, M. J. KARNOVOSKY and M. L. KARNOVOSKY, *Biochem. J.* **116,** 207 (1970).
10. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. Biochem.* **193,** 265 (1951).
11. A. HAKSAR and F. G. PERON, *Biochem. biophys. Acta* **264,** 548 (1972).

Biochemical Pharmacology, Vol. 23, pp. 754–758. Pergamon Press, 1974. Printed in Great Britain.

Substrate stimulation of organic anion transport in newborn dog kidney and choroid plexus*

(*Received* 20 *July* 1973; *accepted* 24 *August* 1973)

THE KIDNEY in the newborn of several species is immature both anatomically[1,2] and functionally.[3] Glomerular filtration rate, renal handling of electrolytes, concentrating and diluting mechanisms and transport of a variety of organic compounds are less in the newborn than in the adult.[3]

Accumulation of *p*-aminohippuric acid (PAH) by renal cortical slices from newborn animals was less than that by slices from adults.[4,5] Treatment of young rats and rabbits with substrates of the organic acid transport system (i.e. penicillin or PAH) enhanced PAH accumulation by renal cortical slices from these animals.[6,7] This stimulation was specific for organic acid transport since base transport, measured by *N*-methylnicotinamide (NMN) accumulation, was unchanged by treatment. Stimulation of PAH accumulation by penicillin appeared to be associated with protein synthesis, perhaps specific transport enzymes.[8,9]

The choroid plexus has been shown *in vitro* to actively accumulate a wide variety of molecules including organic anions.[10–12] The process of choroid plexus transport is believed to be similar to that in renal tubules. Also like the kidney, transport of organic anions by the choroid plexus is immature in the newborn.[11,12] It was reasoned that accumulation of PAH by choroid plexus might be stimulated by penicillin treatment as it is in the kidney.

The purpose of this investigation was to determine the effect of substrate (i.e. penicillin) treatment on organic acid transport in newborn dog kidney and choroid plexus.

Mongrel pups were allowed to stay with the bitch until used for an experiment. Procaine Penicillin G suspension (Duracillin; Eli Lilly & Co., Indianapolis, Ind.) was administered i.m. or s.c. in a dose of 300,000 IU/kg twice daily for 3 days prior to 1 or 2 weeks of age. Control littermates were injected with physiological saline (pH 7·4). Forty-eight hr after the last injection, pups were weighed and killed by decapitation.

The kidneys were rapidly removed, freed of capsule, weighed and placed in iced Ringer-phosphate solution. Renal cortical slices 0·3–0·4 mm thick were prepared freehand. The outer single slices were pooled, incubated together and termed outer renal cortex; deeper slices (second and inward) were pooled and termed inner renal cortex. Duplicate samples of approximately 100 mg of slices were incubated in 2·7 ml of Cross and Taggart phosphate medium[13] in a Dubnoff metabolic shaker for 90 min at 25° under 100% oxygen. The medium contained $7{\cdot}4 \times 10^{-5}$ M PAH and $6{\cdot}0 \times 10^{-6}$ M NMN-^{14}C (4·6 mCi/m-mole). After incubation, slices were quickly removed from the medium, blotted, weighed and analyzed for PAH and NMN as reported previously.[14]

* Supported in part by USPHS Grant AM10913 and NSF Grant GB-32345.