Fox, M., Herve, A., Lazar, J., Rad. Res., 1955, v2, 392.
9. Langendorff, H., Melching, H. J., Strahlenther., 1959, v110, 505.
10. Dukor, P., ibid., 1962, v117, 330.
11. Lohmann, W., Moss, A. J., Sanders, J. L., Porter, B. J., Woodall, D. M., Rad. Res., 1966, v29, 115.
12. Radda, G. K., Biochim. Biophys. Acta, 1966, v112, 448.

Received July 10, 1967. P.S.E.B.M., 1967, v126.

Stimulation of Rat Liver RNA Synthesis by Borate.* (32535)

ULRICH WESER† (Introduced by Harry G. Day)
Department of Chemistry, Indiana University,‡ Bloomington

The essentiality of boron in all plants that have been studied is widely recognized. Investigations regarding the mode of action of borate in plants indicate an involvement in nucleic acid metabolism. Thus RNA synthesis in beans and sunflowers appeared to increase when borate was present(1,2). Since it has not been established whether or not boron is essential in animals(3,4), it seemed to be of importance to study the effect of borate on RNA synthesis in rats. Liver RNA being rapidly synthesized can easily be labeled with [6-^{14}C] orotic acid, a precursor of RNA (5,6). This method allows the determination of even small changes in the rate of RNA synthesis. If borate should be involved in mammalian nucleic acid metabolism, this could be demonstrated by means of this labeling procedure.

Materials and methods. Three groups of male Sprague-Dawley rats, 5 per group with an average initial weight of 37 g, were restricted to a basal diet and environment extremely low in boron.

The basal diet was composed of: Hammarsten casein (Nutritional Biochemicals Corp.) 20%, reagent grade sucrose (Fisher Scientific Co.) 70%, salts 4%, Crisco oil 5%, vitamin mixtures 1%. The salt mixture and vitamin mixtures were essentially the same as used by Maurer and Day(7) in a study of the essentiality of fluorine in nutrition. The concentration of boron was not more than 0.001 ppm (10^{-7} M/kg), as determined using an adaptation of the procedure of Spicer and Strickland(8). The animals received highly deionized water. Groups 1 and 2 were kept on the boron-deficient diet, but Group 3 received the diet to which boric acid was added to provide 1 ppm boron (10^{-4} M/kg).

Six weeks later each animal from each group was injected intraperitoneally with 50 μC of [6-^{3}H]-thymidine (1.9 C/mM, Nuclear Chicago), and 9.5 hours later with 5 μC of [6-^{14}C]-orotic acid (44.5 mC/mM, Nuclear Chicago). However, the rats of Group 2 received simultaneously with each injection 20 μM borate as boric acid. After exactly 10.0 hours the rats were killed by decapitation and the well-bled livers were immediately removed and chilled in ice cold 0.32 M purified sucrose + 3 mM $MgCl_2$. The nuclei were isolated essentially as described by Higashi and Busch(5) and Widnell and Tata (9). The entire operation had to be performed within 24 hours. Aliquot parts (0.2 ml) of the nuclear suspensions were precipitated with 2% perchloric acid. The pellets were carefully washed with perchloric acid and ethanol and finally freeze-dried. To each pellet 0.5 ml hydroxide of Hyamine (obtained from Packard Instrument Co.) was added. After incubation for 6 hours at 37°, each suspension was diluted with 15 ml of a modified scintillation solution(10). Radioactivity was estimated in a liquid scintillation spectrometer (Packard Tri Carb model 3003) and was of the order of 10^3 c.p.m. per sample. The liver nuclei suspensions of each rat were

* Supported by USPHS Grant AM 08209 03 to Harry G. Day

† Present address: Physiologisch Chemisches Institut der Universität, Tübingen, Germany.

‡ Publication no. 1523.

analyzed for their respective contents of RNA and DNA. After they had been extracted with 10% perchloric acid at 70°C RNA was determined using the modified colorimetric procedures of Ceriotti(11) and Drury(12), while the DNA was assayed as described by Burton(13). The results for RNA and DNA synthesis are presented in Tables I and II, respectively. The ±-values are standard deviations. The results were subjected to a "t"-test of significance(14).

TABLE I. Radioactivity of Nuclear RNA After a 30 Min Pulse of 5 μC of [6-^{14}C]-Orotic Acid.

Group	cpm/μg RNA	% Increase
1	48 ± 15	—
2	76 ± 5	58
3	55 ± 8	15

TABLE II. Radioactivity of Nuclear DNA After a 10 Hr Pulse of 50 μC of [6-^{3}H]-Thymidine.

Group	cpm/μg DNA
1	30 ± 22
2	14 ± 4
3	19 ± 13

Results and discussion. The [6-^{14}C]-orotic acid was incorporated to a substantial degree into the liver RNA by all 3 groups (Table I), but the two groups that received borate had higher rates of incorporation than the group that remained deficient. The injected borate (Group 2) caused a highly significant stimulation of RNA synthesis ($p<0.01$) but the effect in animals receiving 1 ppm boron in the diet (Group 3) was only indicative of significance ($p<0.3$). No definite results were obtained in determining the effect of borate on the synthesis of liver DNA as measured by the use of [6-^{3}H]-thymidine injections (Table II). The long time interval after the injections and the high dosage of [6-^{3}H]-thymidine may have contributed to the large deviations within the groups. This is of interest in relation to the observation of Albert(15) that the root tips of tomatoes grown in a boron-deficient medium had less RNA but the DNA did not change.

To exclude effects due to non-specific adsorption or trapping of [6-^{14}C]-orotic acid, all suspensions of the residual nuclei within each group were combined and their RNA isolated(5). The procedure was modified by using bentonite throughout all phenol extractions. The isolated RNA from the various groups was dissolved in water and readings were taken at 260 mμ and 280 mμ with a Gilford spectrophotometer. Finally, all solutions were diluted to give exactly the same extinction at 260 mμ. One ml from each solution was freeze-dried and the radioactivity measured as described previously. The data are presented in Table III. The results obtained with the isolated nuclear RNA (Table III) agree with the data from the radioactive determination of the whole nuclear fractions. Both borate-treated groups showed an increase of RNA synthesis, but again the increase was greater in the rats given an injection of boric acid before they were sacrificed and the livers removed for analysis. The consistency of the effect suggests that boron is biochemically active in the mammalian organism.

TABLE III. Radioactivity of Isolated Nuclear RNA After a 30 Min Pulse of [6-^{14}C]-Orotic Acid.

Group	$\frac{A_{280\,m\mu}}{A_{260\,m\mu}}$	cpm/ml ($A_{260\,m\mu}=0.545$)	% Increase
1	0.496	433	—
2	0.480	602	39
3	0.512	457	6

Very little is known regarding the participation of borate in biochemical reactions involving nucleosides or nucleotides. Thus the stability constants of some boric nucleosides (16,17) are roughly in the same order of magnitude as the formation constants of alkaline earth nucleotide complexes(18). However, these cations are able to affect very strongly some biochemical equilibria by reacting with the base residue or the phosphoric acid moiety of the nucleotide. On the other hand, it is possible that boron-oxy-anions could influence to a certain extent some biochemical reactions by blocking the 2′, 3′-hydroxyl groups of the ribose moiety.

Further investigation is necessary to determine the essentiality of boron in the synthesis of mammalian RNA.

Summary. Young rats on a diet containing not more than 0.001 ppm boron were used to study the effect of administered boric

acid on the synthesis of nuclear liver RNA. A 30-min pulse of [6-^{14}C]-orotic acid resulted in a higher incorporation of ^{14}C in liver RNA when borate was given intraperitoneally. This increased incorporation was diminished when the diet contained 1 ppm boron as boric acid. An attempt to determine the effect of borate on the synthesis of liver DNA, using [6-^{3}H]-thymidine, gave unsatisfactory results. It is apparent that borate increases the rate of synthesis of nuclear liver RNA.

I wish to express my gratitude to Professors Harry G. Day and H. R. Mahler, without whose encouragement and support this work would not have been possible. The technical assistance of Mrs. R. Collins and Mr. D. Perkins is also gratefully acknowledged.

1. Shkol'nik, M. Ya., Maevskaya, A. N., Sclov'eva, E. A., Proc. 5th Intern. Congress of Biochemistry, Moscow, Vol. IX, p. 564, ed. by Sissakian, N. M., Pergamon Press, New York, 1963.

2. Sherstnev, E. A., Kurilenok, G. V., Akad. Nauk SSR Dokl., 1962, v142, 1201.

3. Orent-Keiles, E., Proc. Soc. Exp. Biol. & Med., 1940, v44, 199.

4. Teresi, J. D., Hove, E., Elvehjem, C. A., Hart, E. B., Am. J. Physiol., 1944, v140, 513.

5. Higashi, K., Busch, H., Biochim. Biophys. Acta, 1965, v103, 339.

6. Bresnick, E., Lanclos, K., Gonzales, E., ibid., 1965, v108, 568.

7. Maurer, R. L., Day, H. G., J. Nutr., 1957, v62, 561.

8. Spicer, G. S., Strickland, J. D. H., Analyt. Chim. Acta, 1958, v18, 231.

9. Widnell, C. C., Tata, J. R., Biochem. J., 1964, v92, 313.

10. Hall, T. C., Cocking, E. C., ibid., 1965, v96, 626.

11. Ceriotti, G., J. Biol. Chem., 1955, v214, 59.

12. Drury, H. F., Arch. Biochem. Biophys., 1948, v19, 455.

13. Burton, K., Biochem. J., 1956, v62, 315.

14. Fisher, R. A., Handbook of Chemistry and Physics, 45th ed., p. A-109, Cleveland, 1964, Chemical Rubber Publishing Co.

15. Albert, L. S., Plant Physiol., 1965, v40, 649.

16. Weser, U., Z. Naturforschg., 1967, v22B, 457.

17. ———, Structure and Bonding, 1967, v2, 160.

18. Phillips, R. S. J., Chem. Revs., 1966, v66, 502.

Received July 10, 1967. P.S.E.B.M., 1967, v126.

Induction of Tryptophane Pyrrolase and Tyrosine-α-Ketogluarate-Transaminase in Regenerating Liver of Hypophysectomized Rats.* (32536)

JÜRGEN DREWS† (Introduced by P. K. Bondy)
Department of Medicine, Yale University School of Medicine, New Haven, Conn.

It is now well established that several control mechanisms governing the synthesis of certain enzymes in normal liver are altered or entirely absent in a number of hepatomas (1). In normal liver the synthesis of cholesterol is controlled by a feedback mechanism which responds to the intracellular concentration of cholesterol. The impairment of this control mechanism in several hepatomas leads to a production of cholesterol far exceeding that of the liver cell(2). Pitot and Morris reported that the activity of the enzyme tryptophane pyrrolase which increases in normal liver after administration of cortisol is very low in the slowly growing hepatoma 5123 and does not rise under the influence of cortisol. The tyrosine-α-ketoglutarate-transaminase activity, however, was found to be high in the hepatoma and could not be further stimulated by administration of cortisol(3). In the hepatomas of adrenalectomized animals the activity of the tyrosine transaminase was still twice as high as in normal liver but considerably lower than in the hepatomas of intact animals. Furthermore, administration of cortisol to adrenalectomized animals produced a 2.5-fold stimulation of the tyrosine transaminase activity in the tumor. Pitot and

* This work was supported by a grant from Schering A. G., Berlin and by grant AM 00254-14 from Division of Grants, Nat. Inst. Health, USPHS.

† Present address: Dept. of Medicine, Univ. of Heidelberg, Heidelberg, Germany.