Table 1. Concentration (parts of fatty acid per million parts of sediment) of the branched-chain and straight-chain saturated fatty acids in sediments.

| Fatty acid | BB (9)* | GM (7) | BH (8)† | GRS |
|---|---|---|---|---|
| $iC_{14}$ | 0.97 | 0.4 | 32 | 0.6 |
| $C_{14}$ | 2.6 | 2.9 | 130 | 3.4 |
| $(i + a)C_{15}$ | 3.1 | 0.6 | 61 | 0.5 |
| $C_{15}$ | 1.3 | 1.9 | 76 | 2.0 |
| $iC_{16}$ | 1.3 | 0.4 | 38 | 0.5 |
| $C_{16}$ | 11 | 15 | 560 | 12.2 |
| $(i + a)C_{17}$ | 1 | 0.1 | 13 | n.d.‡ |
| $C_{17}$ | 1 | 1.0 | 39 | 1.6 |
| $iC_{18}$ | 0.84 | n.d. | n.d. | 2.5§ |
| $C_{18}$ | 3.0 | 3.5 | 140 | 8.4 |

* Numbers in parentheses indicate numbers of samples averaged. See text for sample locations. † Calculated on a carbonate-free basis. ‡ Not detected. § Identification tentative.

iso and anteiso acids are about equally abundant. Usually the even-carbon acids have only the iso acid. The iso $C_{12}$ acid and several monounsaturated acids are present although not listed in Table 1. The relatively high ratio of branched-chain acids to straight-chain saturated acid for the various carbon numbers (especially the even ones) raises a problem concerning possible origins.

Sediments receive a small fraction of every type of fatty acid produced by its community. The fatty acid pattern of a sediment will depend (i) on the nature of the acids supplied by the community, and (ii) on the relative survivability, both biological and chemical, of the different acids. Branched-chain acids are chemically as stable as the normal acids and are certainly no more subject to biological degradation than straight-chain acids. Therefore the most important factor is the nature of the acids being produced by the organisms which make up the marine community.

The ratios of the major straight-chain saturated acids to the corresponding branched-chain isomer is lower in sediments than the same ratio is in higher organisms which we have analyzed. This comparison holds for a variety of higher marine organisms reported by Ackman and Sipos (*7*). For their organisms the ratios of straight-chain to branched-chain (iso) for $C_{16}$ and $C_{14}$ are between 100 and 500. The same ratios for sediments are between 1 and 20. This suggests that higher organisms are not the major source of the branched-chain acids in sediments. One should make the same ratio comparisons for phyto- and zooplankton but scarcity of data does not seem to justify doing so. Williams (*8*) studied the fatty acids of six phytoplankton and reported no branched-chain acids. Only minute traces of branched-chain acids were found in ten species of blue-green algae studied in this laboratory. While we do not know the contribution made by plankton to the branched-chain acids in sediments, we would like to suggest a possible source. Bacterial lipids are noted for being rich in branched-chain acids (*9*). Kaneda has shown that the major fatty acids of *Bacillus subtilis* are the iso and anteiso acids (*10*). Two of the five ciliated protozoa studied by Erwin and Bloch (*11*) contained significant amounts of iso acids (23 and 12 percent of the total acids); both bacteria and protozoa live in the upper sediment, deriving food from organic detritus and perhaps producing enough branched-chain acids to account for the enrichment observed.

The branched-chain fatty acids promise to be an interesting group of compounds for organic geochemists. They are present in at least one ancient sediment, the Green River shale (Table 1). It remains to be seen whether they will be found in the variety of ancient sediments which contain straight-chain acids (*1*). The Ponca City crude oil contained 1.8 percent of *n*-decane, 0.3 percent of 2-methylnonane, 0.1 percent of 3-methylnonane, 0.1 percent of 4-methylnonane, and no 5-methylnonane (*12*). The relationship of the iso paraffin being the second most abundant isomer holds for the nonanes and octanes; unfortunately data is not available for the higher paraffins. This correspondence between the methyl branched-chain fatty acids and paraffins suggests either a genetic relationship or simply that organisms make paraffin skeletons much as they make fatty acid skeletons.

RICHARD F. LEO
PATRICK L. PARKER
*Institute of Marine Science, University of Texas, Port Aransas 78373*

### References and Notes

1. J. E. Cooper and E. E. Bray, *Geochim. Cosmochim. Acta* **27**, 1113 (1963).
2. P. H. Abelson, T. C. Hoering, P. L. Parker, in *Advances in Organic Geochemistry*, U. Colombo and G. D. Hobson, Eds. (Pergamon, New York, 1963).
3. P. L. Parker and R. F. Leo, *Science* **148**, 373 (1965).
4. The British Honduras samples were donated by the Phillips Petroleum Co. Dr. T. C. Hoering collected and donated the Green River shale sample.
5. F. P. Woodford and C. M. van Gent, *J. Lipid Res.* **1**, 188 (1960).
6. R. T. O'Connor, in *Fatty Acids*, K. S. Markley, Ed. (Interscience, New York 1960).
7. R. G. Ackman and J. C. Sipos, *Comp. Biochem. Physiol.* **15**, 445 (1965).
8. P. M. Williams, *J. Fisheries Res. Board Can.* **22**, 5 (1965).
9. F. B. Shorland, in *Comparative Biochemistry*, M. Florkin and H. S. Mason, Eds. (Academic Press, New York, 1962), vol. 3, part A.
10. T. Kaneda, *J. Biol. Chem.* **238**, 1122 (1963).
11. J. Erwin and K. Bloch, *ibid.*, p. 1618.
12. W. L. Whitehead and I. A. Breger, in *Organic Geochemistry*, I. A. Breger, Ed. (Pergamon, New York, 1963).
13. Supported by NSF grant GP 2515.

2 February 1966

## Prolonged Immunosuppression and Tumor Induction by a Chemical Carcinogen Injected at Birth

Abstract. *The injection of 60 micrograms of 9,10-dimethyl-1,2-benzanthracene into newborn mice gave rise to a very high incidence of malignant thymomas. The tumor incidence was directly related to the dose of the carcinogen. The neonatal injection of the carcinogen also resulted in a depression in the immune response when the animals were challenged with an antigen as early as 4 weeks or as late as 11 weeks after administration of the carcinogen.*

Neonatal injection of chemical carcinogens into mice induces a variety of tumors (*1, 2*). The strain of mouse used in our experiments (*3*) was extremely sensitive to the induction of malignant lymphomas when the animals were injected with 9,10-dimethyl-1,2-benzanthracene (DMBA) at birth. The number of lymphomas induced was related to the dose of carcinogen. In addition, the neonatal injection of DMBA resulted in a prolonged depression of the immunological response.

A colloidal suspension of DMBA prepared according to the method of Pietra *et al.* (*1*) was injected subcutaneously in the intrascapular region of mice that were less than 24 hours old. Care was taken to prevent leakage of the carcinogen at the injection site. One-half of the mice of each litter were injected with carcinogen and the other half received an equal volume of 1-percent aqueous gelatin. Both treated and control mice were kept together with their mothers for 1 month, at which time they were weaned, separated according to sex, and thenceforth observed for the appearance of tumors.

Our results show that the number of lymphomas induced is related to the dose of carcinogen. Of 120 mice injected with 60 $\mu$g DMBA, 90 percent developed lymphomas, whereas the tu-

Table 1. The effect of variation in the length of time between carcinogen and antigen treatment. The numbers in the parentheses represent the number of animals tested. Results are $\log_2$ of average hemolysin titer.

| Time after immunization (days) | Interval between carcinogen and antigen (weeks) | | | |
|---|---|---|---|---|
| | Group | 4 | 7–9 | 11 |
| 4 | Treated | 6.0(7) | 6.6(8) | 4.8(5) |
| 4 | Control | 8.0(6) | 9.8(10) | 8.3(6) |
| | | $P<.001$ | $P<.001$ | $P<.001$ |
| 10 | Treated | 6.3(3) | 4.6(8) | 4.8(8) |
| 10 | Control | 7.0(3) | 7.0(10) | 6.8(8) |
| | | | $P<.001$ | $P=.02$ |
| 19 | Treated | | 3.5(8) | |
| 19 | Control | | 6.9(10) | |
| | | | $P<.001$ | |
| 30 | Treated | 4.3(9) | | 4.4(7) |
| 30 | Control | 7.0(7) | | 7.7(8) |
| | | $P<.001$ | | $P=.002$ |
| 60 | Treated | 4.1(8) | | 4.3(3) |
| 60 | Control | 6.6(7) | | 6.5(4) |
| | | $P<.01$ | | |

mor incidence was only 53 percent for 76 mice injected with 30 $\mu$g and 19 percent for 47 mice receiving 10 $\mu$g. The incidence for mice receiving gelatin only was 4 percent. The first lymphomas began to appear approximately 10 weeks after treatment, and by 25 weeks virtually all mice that were going to develop a thymoma had done so. The three lymphomas that arose in 73 gelatin-injected control animals did not appear until 43 weeks, or more, after injection.

The whole-body weights of treated and control newborn animals were followed for 7 days. No differences were detected, a contrast to the results of Rappaport *et al.* (*4*) who observed a decrease in the whole body weight 4 days after the injection of 100 $\mu$g of DMBA.

To determine what tissues were being affected by the carcinogen treatment, the ability of the lymphatic system to respond to an antigenic stimulus was investigated. Mice, less than 24 hours old, received 60 $\mu$g of DMBA or, in the controls, 1 percent gelatin, and beginning at 4 weeks of age they were challenged with an antigen at various intervals. The preparation of the antigen and hemolysin assays have been reported (*5*). All titers are expressed as the logarithm to the base 2 (log 2) of the dilution (Table 1). Thus, a small dose of DMBA given within the first 24 hours after birth produced a significant and long-lasting reduction of the ability of the treated animals to respond to an antigenic stimulus when challenged as early as 4 weeks or as late as 11 weeks after carcinogen treatment.

A further attempt was made to obtain information on the site of action of DMBA with respect to its effect on the immunological response. It had been shown (*6*) that mice splenectomized as adults and challenged with a low dose of antigen administered intravenously do not give a detectable antibody response. Intraperitoneal injection of the antigen to splenectomized animals resulted in a response arising only from the extrasplenic lymphatic tissue. If splenectomy does not alter the response of the extrasplenic tissues, then it is possible to obtain information on the portion of the antibody response contributed by the extrasplenic tissue and, by difference, an estimate of the contribution of the spleen to the response. The results of this experiment indicate that 89 percent of the total response was contributed by the spleen as compared to 11 percent by the extrasplenic tissues. However, a comparison of the effect of 60 $\mu$g of DMBA on the responding tissues showed that both are affected proportionately to the same extent.

Malmgren *et al.* (*7*) showed that very large doses of chemical carcinogens injected into adult mice were able to depress the immunological response when the animals were challenged with an antigen administered during carcinogen treatment. The noncarcinogenic chemicals tested had no effect on the immunological response. Stjernswärd (*8*) has shown that 3-methylcholanthrene given in a large, single dose to adult animals 6 days before antigenic stimulation, not only depressed the hemolysin response but also resulted in a decrease in the number of antibody-forming spleen cells. Prehn and Main (*9*) have suggested that the impairment of the immunological response of the host is related to the ability of 3-methylcholanthrene to act as a carcinogen.

Thus, neonatal injection of the potent carcinogen DMBA depresses the capacity of the animals to produce hemolysins to sheep's erythrocytes, and it does not matter when the erythrocytes are given: the immunological capacity of the animal is chronically impaired throughout the duration of the latent period for the development of the thymomas. Possibly such chronic impairment plays an important role in carcinogenesis (*10*).

J. K. BALL*
N. R. SINCLAIR
J. A. MCCARTER*
*Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia*

### References and Notes

1. G. Pietra, K. Spencer, P. Shubik, *Nature* **183**, 1689 (1959).
2. B. Toth, H. Rappaport, P. Shubik, *J. Nat. Cancer Inst.* **30**, 723 (1963).
3. J. K. Ball, T. Y. Huh, J. A. McCarter, *Brit. J. Cancer* **18**, 120 (1964).
4. H. Rappaport and C. Baroni, *Cancer Res.* **22**, 1067 (1962).
5. N. R. Sinclair, *Nature* **208**, 1104 (1965).
6. D. A. Rowley, *J. Immunol.* **64**, 289 (1950); F. L. Adler, *ibid.* **95**, 26 (1965).
7. R. A. Malmgren, B. E. Bennison, T. W. McKinley, *Proc. Soc. Exp. Biol. Med.* **79**, 484 (1952).
8. J. Stjernswärd, *J. Nat. Cancer Inst.* **35**, 885 (1965).
9. R. T. Prehn and J. M. Main, *J. Nat. Cancer Inst.* **18**, 769 (1952).
10. M. C. Berenbaum, *Brit. Med. Bull.* **20**, 159 (1964).
11. Supported by grants from the National Cancer Institute of Canada and by a Medical Research Council of Canada fellowship (N.R.S.) and scholarship (J.K.B.). We thank Mrs. L. Stewart for valuable technical assistance.
* Present address: Cancer Research Laboratory, University of Western Ontario, London, Ontario. Requests for reprints should be sent to this address.

21 January 1966

## Enzyme Amplifier Kinetics

Abstract. *Enzyme amplifier systems have been implicated in such diverse physiological phenomena as vision and blood coagulation. Such systems are considered from a general point of view and the concept of steady state gain of the enzyme amplifier is introduced. Expressions for the latter are obtained, and several of the main factors influencing gain are discussed. The effect of the duration of the activation on the characteristics of the response is discussed, and the influence of small changes in the rate constants on the steady state gain is considered.*

In recent years it has been suggested that certain important biological processes involve a sequence of enzyme-proenzyme reactions which exhibit a net chemical gain. Thus, MacFarlane (*1*) has proposed that blood clotting constitutes such an enzyme sequence. In this instance surface activation of comparatively few molecules of Hageman factor serves to activate further a series of some six additional factors which culminates in the conversion of millions of molecules of fibrinogen to fibrin. Wald (*2*) has also considered the possibility that vision may involve enzyme amplification since the