Technical Papers

The Effect of 2-Aminofluorene on the Utilization of S^{35} DL-Methionine[1]

A. W. Wase, J. B. Allison, and J. F. Migliarese
Bureau of Biological Research, Rutgers University, New Brunswick, New Jersey

Impaired utilization of protein by rats and dogs fed 2-acetyl-aminofluorene has been demonstrated together with the protective effects of dietary riboflavin (*1–5*). Studies have been planned to determine the effects of this carcinogen on the utilization of some of the essential amino acids. The following is a preliminary report involving S^{35} DL-methionine.

The experiments involved both *in vivo* and *in vitro* studies of the uptake of S^{35} DL-methionine by the livers of rats. The *in vitro* experiments were planned as follows:

Livers from normal rats were sliced and perfused in cold Krebs saline. The slices were removed, blotted dry, and placed in a medium consisting of Krebs-buffered saline containing 30 mg% S^{35} DL-methionine with a specific activity of 167 c/m/mg DL-methionine. The system was incubated at 37° C for 2 hr in an air atmosphere.

Similar systems were employed, including 2-aminofluorene in concentrations ranging from 19.6 to 98.0 μg AF · HCl/100 ml buffered saline, with and without riboflavin in several progressive concentrations. Following the incubation period, slices were removed, washed, blotted dry, digested in HNO_3, and an aliquot was assayed for radioactivity. Results are expressed in terms of μg of methionine incorporated per gram of liver slices.

The *in vivo* experiments were carried out as follows: Two groups (12 each) of adult (200–250 g) male Wistar rats were fed the basal 18% casein diet (*4*), one group receiving .03% of the carcinogen in the diet. The animals were fed *ad lib* for a period of 56 days. At the end of this experimental period each animal was given 3 mg S^{35} DL-methionine intraperitoneally. After 24 hr, half the animals in each group were killed and the livers removed. Livers were washed in cold saline, blotted dry, and weighed. Samples of approximately ½ g were taken for radioactivity assay. After 72 hr the remaining rats were autopsied and treated as above.

Previous studies have demonstrated that aminofluorene compounds inhibit the growth and utilization of nitrogen by animals (*1–5*), an inhibition which is overcome by riboflavin. The data in Table 1 illustrate another example of inhibition by 2-aminofluorene overcome by this vitamin.

The incorporation of S^{35} DL-methionine in liver slices is decreased markedly by the presence of AF · HCl. This inhibition is reversed and the uptake returned to normal with the introduction of riboflavin into the system. It is possible that the addition of 2-aminofluorene may alter intermediary metabolic processes involving methionine, thereby depleting the animal in protein stores (a depletion), which is overcome with riboflavin. This possibility is under investigation.

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TABLE 1

EFFECT OF VARYING 2-AMINOFLUORENE (AF) AND RIBOFLAVIN ON THE UPTAKE OF S^{35} DL-METHIONINE IN RAT LIVER SLICES

2-Aminofluorene (μg/ml)	Riboflavin (μg/ml)	Uptake (% of controls)
0.000	0.00	100.0
0.05	—	68.3
0.10	—	56.0
0.20	—	42.0
0.40	—	31.5
1.0	—	31.1
0.05	0.06	79.3
0.05	0.13	99.6
0.20	0.18	101.0
0.20	0.24	98.0
0.20	4.00	99.2
—	0.06	99.2
—	0.13	106.5
—	0.24	102.7
—	1.17	105.0
—	4.00	98.0

Animals with depleted protein stores exhibit an increased tendency to utilize and retain dietary amino acids, an increase in protein anabolism that is associated with growth and regeneration (*6*). It is interesting to note that the amount of S^{35} DL-methionine taken up by the liver in 24 hr is greater in animals fed the carcinogen for 56 days (Table 2), although this greater uptake is followed in the next 48 hr by a more rapid loss than normal. The enzymatic significance of this greater uptake and utilization of S^{35}

TABLE 2

UPTAKE (24 HR) AND UTILIZATION (72 HR) OF METHIONINE (CALCULATED FROM THE ACTIVITY OF S^{35}) IN LIVERS OF RATS FED DIET WITH OR WITHOUT 2-ACETYL AMINOFLUORENE FOR 56 DAYS. EACH VALUE AN AVERAGE ON 6 ANIMALS

	Methionine			
	24 hr	72 hr	24 hr	72 hr
Diet	μg/liver		μg/liver/100 g body wt	
18% Casein	142 ± 11	87 ± 9	558 ± 42	317 ± 29
18% Casein + 0.03% 2-acetyl aminofluorene	180 ± 13	78 ± 12	1165 ± 59	472 ± 33

DL-methionine in animals treated with carcinogen is under investigation. It is thought possible that increased utilization of S^{35} DL-methionine may be associated with a greater potential for growth or regeneration of tissues, a hypothesis that is being tested in normal and protein-depleted rats.

References

1. WASE, A. W., and ALLISON, J. B. *Proc. Soc. Exptl. Biol. Med.*, **73**, 147 (1950).
2. ALLISON, J. B., *et al. Cancer Research*, **10**, 266 (1950).
3. WASE, A. W., and ALLISON, J. B. Abstr., *119th Meeting Am. Chem. Soc.* (April 1951).
4. WILSON, R. H., DEEDS, F., and COX, A. J. *Cancer Research*, **1**, 595 (1941).
5. BIELSCHOWSKY, F., and GREEN, H. N. *Nature*, **149**, 526 (1942).
6. WASE, A. W., ALLISON, J. B., and MIGLIARESE, J. F. *Federation Proc.*, **11**, 307 (1952).

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On the Anomaly in the Heat Capacity of Manganous Oxide[1]

Roald K. Wangsness

U. S. Naval Ordnance Laboratory, White Oak, Maryland

Recent work of Todd and Bonnickson (*1*) on very pure samples of MnO shows that as the temperature increases in the range from 100° to 122° K the heat capacity of MnO rises from about 7 cal/(mole deg) to a peak of 76.7 and then drops sharply to about 7.5. The total heat absorbed is 246 cal/mole. The average Debye heat capacity is about 7, so that $7 \times 22 = 154$ cal/mole are absorbed by lattice oscillations. One can estimate the additional heat capacity associated with the disappearance of the spontaneous magnetization to be R (*2*), so that the heat ascribable to this effect is 44 cal/mole. Thus, there is an amount of heat $q = 48$ cal/mole (nearly 20% of the total) which cannot be accounted for by the usual mechanisms.

MnO is antiferromagnetic with $\theta = -610°$ K and $T_c \simeq 122°$ K, where θ and T_c are the characteristic temperature of the Curie-Weiss law and the Curie temperature, respectively (*3*). The fact that $-\theta/T_c$ is equal to 5 rather than unity, as predicted by the simple theory, has been discussed by means of an extension of the molecular field theory to include the effects of nearest and next-nearest neighbor interactions in more complicated orderings of the elementary moments contained in the magnetic sublattices (*4*). If γ_1 and γ_2 are the molecular field coefficients for nearest and next-nearest neighbor interactions, respectively, then the ratio $-\theta/T_c$ for MnO determines $\rho = \gamma_2/\gamma_1$, to be 0.75; this value is compatible with two different orderings known as ordering of the second or third kind, which can occur if ρ is greater or less than 3/4, respectively. Neutron diffraction experiments have shown the ordering below the Curie temperature to be of the second kind (*5*).

It has been found (*6*) that the lattice constants of MnO change in this temperature range, and since γ_1 and γ_2 (and thence ρ) presumably depend strongly upon distance, it is suggested that the anomalous behavior can be further interpreted as a change from the second to the third kind of order as the temperature is increased. To correspond to the neutron diffraction results, we should have $\rho = (3/4) + \delta$ below the Curie temperature, and $\rho = (3/4) - \varepsilon$ throughout the transition region to conform to this suggestion (δ and ε are small positive numbers). The following calculations provide some quantitative support for this interpretation.

If T_i is the Curie temperature for the ith kind of ordering, one finds that

$$T_2 = C\,\rho\,\gamma_1,\ T_3 = C\,\gamma_1\,[1 - (1/3)\,\rho],$$

and

$$-\theta = 3C\,\gamma_1\,[1 + (1/3)\,\rho]$$

where C is the Curie constant (*4*). A reasonable expression for the range ΔT of the anomalous behavior is $T_3 - T_2$, so that we obtain

$$\Delta T = -(\theta/3)\left(1 - \frac{4}{3}\rho\right)\left(1 + \frac{1}{3}\rho\right)^{-1} \simeq (16/9)\,T_c\varepsilon.$$

Using the experimental values of ΔT and T_c, we find the estimated value of ε to be 0.1. This corresponds to a 13% change in ρ which is satisfactorily small considering the nature of the calculation.

The energy which must be supplied to the system when in the ith kind of ordering in order to destroy the magnetization is of the order of RTi. Thus, for the heat absorbed during the change of ordering, we can write

$$q = R(T_3 - T_2) = (16/9)RT_c\varepsilon.$$

From this we find that $q = 43$ cal/mole, which agrees quite well with the value of 48 given above.

References

1. TODD, S. S., and BONNICKSON, K. R. *J. Am. Chem. Soc.*, **73**, 3894 (1951).
2. LI, Y. Y. *Phys. Rev.*, **84**, 721 (1951).
3. BIZETTE, H. *Ann. phys.*, **1**, 306 (1946).
4. NÉEL, L. *Ibid.*, **3**, 137 (1948); ANDERSON, P. W. *Phys. Rev.*, **79**, 705 (1950); VAN VLECK, J. H. *J. phys. radium*, **12**, 262 (1951); SMART, J. S. *Phys. Rev.*, **86**, 968 (1952).
5. SHULL, C. G., STRAUSER, W. A., and WOLLAN, E. O. *Phys. Rev.*, **83**, 333 (1951).
6. RUHEMANN, B. *Phys. Z. USSR*, **7**, 590 (1935); TOMBS, N. C., and ROOKSBY, H. P. *Nature*, **165**, 442 (1950).

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Ammoniated Dentifrices and Hamster Caries: Further Studies on the Effects of Ingestion[1]

David F. Mitchell, Edith Zak Helman, and Dwight S. Chernausek

School of Dentistry, University of Minnesota, Minneapolis

Controversy exists concerning the efficacy of ammoniated dentifrices in dental caries prevention. *In*

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