Summary. A substantial proportion (47%) of apparently healthy old mice possessed significantly greater numbers of spleen cells capable of autoimmune hemolytic plaque production than did middle-aged or young adult mice of the same genotype. The average plaque counts obtained with old (C57Bl/10 × B10.LP)F1 male mice 4 to 5 times higher than those obtained with comparable younger mice. This evidence supports earlier suggestions of a meaningful association between autoimmunity and aging.

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Inhibition of Adenosine Deaminase by a Metabolite of the Nephrotoxic Drug, Puromycin Aminonucleoside.* (31503)

N. DICKIE,† L. NORTON, R. F. DERR, C. S. ALEXANDER, AND H. T. NAGASAWA

Medical Research Laboratories, Minneapolis Veterans Hospital, and Departments of Medicine and Pharmaceutical Chemistry, University of Minnesota, Minneapolis

Administration of the aminonucleoside of puromycin (PA)‡ to the rat produces a nephrotic syndrome characterized by proteinuria, hypoalbuminemia, hypercholesterolemia, edema, and ascites(1-4). The principal necleoside metabolite in 8-hour rat urine after PA administration is the monodemethylated analog, MMPA(5). Previous studies by Cory and Suhadolnik(6) showed that the didemethylated analog of PA, APA, is a substrate for adenosine deaminase (E. C. 3.5.4.4) and that N^6-methyladenosine is an inhibitor of the enzyme. Model studies by Schaeffer *et al*(7) suggested that MMPA would be an inhibitor of adenosine deaminase. The present studies demonstrate that MMPA does indeed inhibit adenosine deaminase.

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† Present address: Food and Drug Directorate, Ottawa, Ont., Canada.

‡ Abbreviations: PA, 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)purine; MMPA, 6-methylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)purine; APA, 6-amino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)purine.

Materials and methods. Calf intestinal mucosal adenosine deaminase, Type II, and N^6-methyladenosine were purchased from the Sigma Chemical Co.

The monodemethylated analog of PA, MMPA, was prepared biosynthetically by incubation of PA with fortified rat liver microsomes according to a modification of the procedure of Axelrod(8). PA (55.6 mg) was incubated at 25° with the microsomes from 10 g of liver together with the requisite cofactors (8) for 4 hours with stirring. The reaction was stopped by heating in a boiling water bath for 10 minutes, and the aminonucleosides isolated by ion-exchange chromatography according to Wilson *et al*(9). The aminonucleosides were separated by chromatography on Whatman 3 MM paper with n-propanol-1 N NH_4OH (75:25, v/v), corresponding to Wilson's solvent system "C"(9). The MMPA band eluted with water was repurified by thin-layer chromatography on Silica Gel HF (Brinkmann) with a chloroform-methanol-2.5 N ammonia (150:100:15, v/v), (CMN) solvent. After elution from the thin-layer

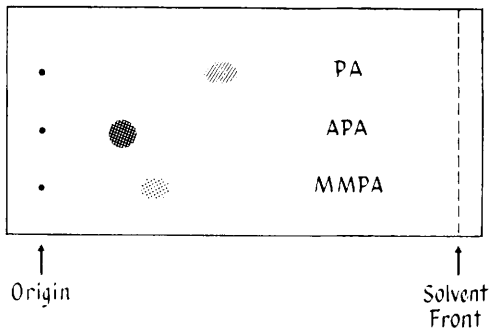

FIG. 1. Thin-layer chromatogram of demethylated aminonucleoside. Absorbent: Silica Gel HF. Solvent: chloroform-methanol-2.5 N ammonia (200:100:15, v/v). Visualization: UV light.

plate with water the MMPA was refractionated on the ion-exchange system to remove some impurities picked up from the thin-layer absorbent.

The structure of the liver microsomal metabolite was established as MMPA thus: PA is known to be demethylated by rat liver microsomes under these conditions(10); the compound behaves like an aminonucleoside on ion-exchange chromatography; upon paper chromatography on Whatman 1 with Wilson's solvent "C" the compound had the same R_F and UV spectra as that reported by Wilson (9) for MMPA; upon thin-layer chromatography on Silica Gel HF with CMN solvent the compound had a mobility intermediate between PA and APA (Fig. 1); acid hydrolysis of the compound with N HCl at 100° for 1 hour gave 6-methylaminopurine and a compound identical to synthetic 3-amino-3-deoxy-D-ribose prepared according to Baker *et al* (11) in the ratio MMPA:6-methylaminopurine:aminoribose of 1:0.81:0.89.

Adenosine deaminase activity was assayed at 30.0° in a final volume of 3.0 ml of 0.01 M Tris (pH 7.1 for MMPA, pH 7.5 for N^6-methyladenosine). Rate of reaction was determined by measuring the appearance of product, inosine, with a Beckman DU spectrophotometer at 240 mμ, or the disappearance of substrate at 265 mμ. The extinction coefficients used were: $E^{mM}_{240} = 3.83$ (12) for inosine and $E^{mM}_{265} = -7.04$(12) for N^6-methyladenosine. The initial reaction velocity was determined graphically from a plot of absorbance *vs* time.

Results and discussion. Inhibition of adenosine deaminase by MMPA. Adenosine concentration was varied from 0.024 to 0.064 mM and the inhibitor (MMPA) concentration was varied from 0 to 0.06 mM. The observed data were exaluated by the method of least squares from a Lineweaver-Burk reciprocal plot(13) (Fig. 2). The inhibition was competitive with $K_m = 4.1 \times 10^{-5}$M and $K_i = 6.5 \times 10^{-5}$M $\pm$ 0.5×10^{-5}M (SEM) at pH 7.1. Implicit in this analysis is the assumption that the amount of bound inhibitor is negligible compared with the amount of free inhibitor. If this were not the case a misinterpretation of the data might be made (14). To obtain evidence that this interpretation (competitive inhibition) was correct, the inhibition by N^6-methyladenosine was more extensively studied.

Inhibition by N^6-methyladenosine. The conditions of the assay (except pH) and the inhibitor concentrations were the same as with MMPA. In this case $K_m = 5.5 \times$

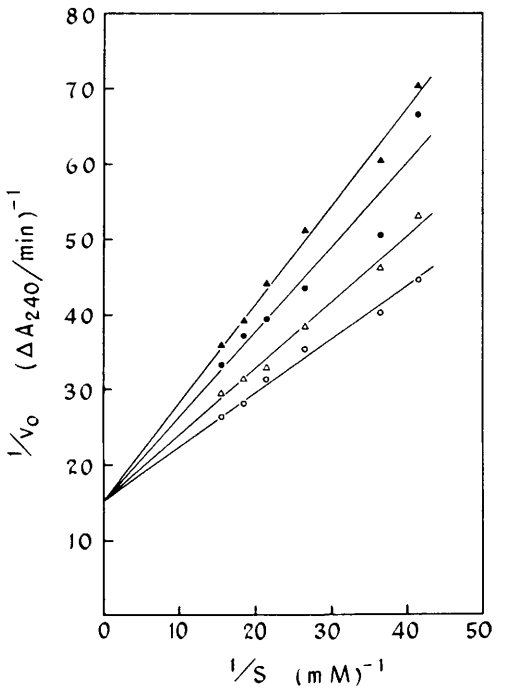

FIG. 2. Lineweaver-Burk plot to determine K_m and K_i of adenosine deaminase at pH 7.1. The inhibitor MMPA was used at the following concentrations: 0 = no inhibitor, △ = 0.02 mM, ● = 0.04 mM, ▲ = 0.06 mM.

10^{-5}M and $K_i = 1.0 \times 10^{-5}M \pm 0.1 \times 10^{-5}M$ (SEM) at pH 7.5, compared with $K_i = 0.53 \times 10^{-5}$ reported by Cory and Suhadolnik(6) using Sigma Type I enzyme at pH 7.0. The data were subjected further to more subtle analysis according to Reiner (14). The inhibition was found to be the "complete exclusive E" type, which corresponds to classical competitive. K_3 of Reiner, which is the inhibitor-enzyme dissociation constant, was 0.9×10^{-5}M which agreed well with K_i. Thus, N^6-methyladenosine is a competitive inhibitor of adenosine deaminase.

Because N^6-methyladenosine and MMPA are structurally similar and their K_is are of the same order of magnitude, the kinetic analysis of one may be related to the other by analogy. Thus, the initial conclusion based on the interpretation of Lineweaver-Burk plots that MMPA is a competitive inhibitor of adenosine deaminase appears to be valid.

The relationship, if any, between the inhibition of adenosine deaminase inhibition by MMPA and aminonucleoside nephrosis is not clear at this time.

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Relationship of Alkaline Phosphatase Levels in Intestinal Mucosa to ABO and Secretor Blood Groups.* (31504)

D. C. SHREFFLER (Introduced by J. V. Neel)
Department of Human Genetics, University of Michigan, Ann Arbor, Mich.

Since the first discovery of a slowly-migrating serum alkaline phosphatase component on starch gel electrophoresis(1,2), it has been abundantly demonstrated that the ABO and Secretor loci influence the occurrence and strength of this slow-moving (B) phosphatase band(2-7). The band is weak or absent in type A individuals, and is absent almost without exception in ABH non-secretor individuals. In type O or type B individuals who are ABH secretors, the B phosphatase component may or may not be present, and if present may vary in concentration over a wide range; this variation is influenced by genetic as well as non-genetic factors(4,5). Similar blood group-associated serum alkaline phosphatase variation has been reported in cattle(8) and in sheep(9).

There is now a compelling body of evidence to indicate that the serum B phosphatase is the same enzyme as a slowly-migrating component found in extracts of intestinal mucosa. Both enzymes have the same electrophoretic mobility on starch gel, as shown by the results of Hodson *et al*(10), and confirmed in our studies. Both enzymes are similarly in-

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