Calcd. for $C_{12}H_{18}BrNO_2S$: C, 46.45; H, 2.60. Found: C, 46.41; H, 2.70.) Reduction furnished 2-amino-2′-bromodiphenyl sulfide, m.p. 62–63°. (*Anal.* Calcd. for $C_{12}H_{10}BrNS$: C, 51.44; H, 3.60. Found: C, 51.00; H, 3.63. Hydrochloride, m.p. 132–134°. *Anal.* Calcd. for $C_{12}H_{11}BrClNS$: C, 45.51; H, 3.50. Found: C, 45.57; H, 3.79.) Benz-*o*-chloroanilide[6] and *o*-chlorophenylacetone[7] were made by standard methods. Acetoacet-*o*-chloroanilide was the product of Union Carbide Chemicals Co. Products were identified by mixed melting points with authentic samples or by comparison of melting points of the product and at least one derivative thereof with literature values. The yields reported are not considered to be optimum.

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(7) I. B. Johns and J. M. Birch, THIS JOURNAL, **60**, 919 (1938).

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RECEIVED MARCH 21, 1958

THE SITE OF CLEAVAGE OF *MYO*-INOSITOL BY PURIFIED ENZYMES OF RAT KIDNEY

Sir:

We have reported previously[1] the cleavage and conversion of inositol to glucuronic acid (racemic mixture) by rat kidney extracts. The enzyme preparation has now been purified, and the system that forms D-glucuronate has been obtained free of the one that forms the L-isomer. Resolution was obtained by treatment with calcium phosphate gel which adsorbs only the system that forms the L-isomer. The enzyme system forming the D-isomer was further purified 200-fold. Attempts to elute the adsorbed enzyme from the gel produced inactive preparations. Employing inositol-2-C^{14} [2] as the substrate and incubating separately with either the crude enzyme system or the purified enzyme capable of yielding only the D-isomer, we isolated 8 to 10 mg. of the respective radioactive glucuronic acids. They were converted to their lactones with m.p. of 176–178° for the racemic lactone[1] and 180° for the D-glucuronolactone having an $[\alpha]^{23}D$ +18.6° (*c* 1 in H_2O, *l* = 1 dm.). The radioactive glucuronolactones were diluted fivefold with the respective non-radioactive glucuronolactones and crystallized to a constant specific activity of approximately 1755 c.p.m. per micromole. The distribution of C^{14} within each of the glucuronates was obtained as follows: the uronic acids were reduced with $NaBH_4$ to gulonic acids which were converted to their lactones. The lactone derived from the racemic glucuronate was optically inactive and could not be crystallized. The lactone of the D-isomer had a m.p. of 184–185° and an $[\alpha]^{23}D$ +56° as expected for L-gulonolactone (*c* 1 in H_2O, *l* = 1 dm.). The gulonolactones were titrated with NaOH and were oxidized with HIO_4 to 1 mole of formaldehyde, 1 mole of glyoxylate and 3 moles of formate per mole of gulonolactone. The formaldehyde was isolated as the dimedon derivative, m.p. 191°, in 95% yield. The formic acid was distilled and isolated as the sodium salt in 100% yield. The glyoxylate was obtained in 85% yield and was characterized as described earlier[3] and by its 2,4-dinitrophenylhydrazone, m.p. 192°. Aliquots of these fractions were plated and counted in a gas flow counter. More than 98.5% of the C^{14} of the degraded gulonolactones were recovered in the glyoxylate fraction. The radioactive glyoxylates were further oxidized with HIO_4 to equimolar amounts of CO_2 and formate.[3] This formate contained all the C^{14} of the glyoxylate. These results demonstrate that the C-2 of inositol becomes C-5 of the racemic as well as of the D-glucuronate. Thus, inositol cleavage by the purified enzyme system occurs between C-1 and C-6 to form *exclusively* D-glucuronate. The formation of L-glucuronate may result from cleavage of inositol between C-3 and C-4, assuming that no racemization occurs of a possible intermediate between inositol and D-glucuronate. Further studies are needed to confirm this latter mechanism.

(1) F. C. Charalampous and C. yras, *J. Biol. Chem.*, **228**, 1 (1957).
(2) We are grateful to Dr. Laurens Anderson for a generous gift of inositol-2-C^{14}.
(3) F. C. Charalampous, *J. Biol. Chem.*, **225**, 585 (1957).

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IDENTIFICATION OF CYANOACETIC ACID AS A METABOLITE OF β-AMINOPROPIONITRILE (BAPN) AND OTHER NITRILES[1]

Sir:

The administration of β-aminopropionitrile (BAPN) to rats or rabbits has been shown to result in the formation of a metabolite which could be detected in the urine or blood serum by an orange-pink spot on paper chromatograms developed with diazotized sulfanilic acid.[2] Efforts to isolate the metabolite from ethyl acetate extracts of acidified urine led to the separation of a colorless, crystalline, nitrogenous solid which was very soluble in water, highly acidic, distillable *in vacuo*, and exhibited the characteristic chromatographic behavior.[2] Further examination of this solid showed that it was not pure, but the metabolite was obtained from it in substantially pure form by ion-exchange chromatography on Dowex-1 resin (acetate form) and has now been identified as *cyanoacetic acid*. The isolated substance melted at 67–68°, undepressed by admixture with an authentic sample, and corresponded closely with known cyanoacetic acid in its infrared spectrum and chromatographic behavior on paper. *Anal.* Calcd. for $C_3H_3O_2N$: C, 42.36; H, 3.56; N, 16.47. Found: C, 42.33; H, 3.68; N, 15.42.

Cyanoacetic acid was also detected in the urine of rats following administration of 3,3′-iminodipropionitrile,[3,4] ethylene cyanohydrin and valeronitrile. Administered cyanoacetic acid was ex-

(1) Supported in part by grants A538(C8) and A1498 from the National Institutes of Health, U. S. Public Health Service.
(2) J. T. Garbutt, J. J. Lalich, S. H. Lipton and F. M. Strong, *Federation Proc.*, in press.
(3) J. Delay, P. Pichot, J. Thuillier and J. P. Marquiset, *Compt. rend. soc. biol.*, **146**, 533 (1952).
(4) H. A. Hartmann and H. F. Stich, *Science*, **125**, 445 (1957).

creted unaltered. Aminoacetonitrile, 2,2′-iminodiacetonitrile and γ-aminobutyronitrile failed to give rise to cyanoacetic acid. It appears that in the metabolism of BAPN and certain other aliphatic nitriles by the rat the organic cyano group is largely unaffected and that deamination and/or oxidation occur at the other end of the molecule. Results to date tend to indicate that aliphatic nitriles are metabolized in the animal body to form ω-carboxynitriles which then undergo cleavage as proposed for the fatty acids.[5] The color reaction with diazotized sulfanilic acid affords a very sensitive method for detecting cyanoacetic acid and may be useful for studying the metabolism of other organic cyano derivatives. A detoxication product of *inorganic* cyanide, 2-imino-4-thiazolidine carboxylic acid,[6] gives a somewhat similar color with this reagent but is easily distinguished from cyanoacetic acid. Neither cyanoacetic acid or cyanacetamide produced any of the symptoms of BAPN toxicity when fed to weanling rats at the rate of 2 mg. per ml. of drinking water for seven weeks.[7] Cyanoacetic acid may be a detoxication product of BAPN. Its relationship, if any, to the connective tissue degeneration characteristic of BAPN toxicity is not known.

(5) D. E. Green, *Biol. Revs.*, **29**, 330 (1954).
(6) J. L. Wood and S. L. Cooley, *J. Biol. Chem.*, **118**, 449 (1956).
(7) J. J. Lalich, unpublished experiments.

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RECEIVED MARCH 6, 1958

UO_2–PuO_2 SOLID SOLUTIONS[1]

Sir:

An X-ray diffraction investigation has been made of the solid phase relationships in the UO_2–PuO_2 system. The results show that a continuous solid solution exists, and that the lattice parameter varies essentially linearly with composition. A slight negative deviation from linearity may exist.

Some trouble was experienced in preparing satisfactory samples of the mixed oxide. Plutonia is an extremely inert substance, and at 1000° the reaction rate between PuO_2 and UO_2 is very slow. Various ways of producing an intimate mixture of plutonium and uranium compounds that could be converted to oxide were tried. The method which seemed best was to co-precipitate $Pu(OH)_4$ and $(NH_4)_2U_2O_7$ by dropping a Pu(IV)–U(VI) solution (total metal concentration *ca.* 14 g./l.) into hot ammonium hydroxide, taking care to keep the *p*H always above 8. The mixed precipitate was dried in air at 70°, then fired in hydrogen by raising the temperature to 1000° over 6 or 7 hours and holding at 1000° overnight. Samples were furnace-cooled in hydrogen.

It appears to be quite important that the firing be done in such a way as to avoid the production of well crystallized PuO_2 as a separate phase. For example, a solid solution was not produced by initial firing of the hydroxide precipitate in air followed by hydrogen reduction of the U_3O_8 to UO_2, nor by hydrogen firing of mixtures produced by evaporation of various solutions containing U and Pu. Coprecipitation of the U and Pu as peroxide was also tried and was not successful.

After the samples were examined by X-ray diffraction, a radiochemical Pu assay was done to check the composition. The Pu assay agreed with the compositions as made in each case.

X-Ray powder patterns were taken in a 114.6 mm. diameter powder camera using filtered copper K-radiation. (Wave lengths, $\alpha_1 = 1.54051$, $\alpha_2 = 1.54433$, mean $\alpha = 1.5418$ Å.) The lattice parameter was determined in most cases by graphical extrapolation of the values found for the high-angle lines on each pattern. Results are presented in Table I. The error limits on the compositions are estimated, those on the lattice parameters are estimated uncertainties in the graphical extrapolation. The solid solution has the fluorite structure, the same as PuO_2 and UO_2.

TABLE I

UO_2–PuO_2 SOLID SOLUTION FLUORITE STRUCTURE

Compn. (mole % PuO_2)	Lattice parameter (Å., 25°)
0	5.4700 ± 0.0001
12.6 ± 0.3	5.449 ± .003
20.0 ± .3	5.4544 ± .0006
35.9 ± .3	5.441 ± .003
48.1 ± .3	5.4345 ± .0005
63.1 ± .3	5.420 ± .003
73.8 ± .3	5.414 ± .002
79.4 ± .3	5.407 ± .003
100.0	5.3960 ± .0003

Thanks are due the Misses Marian Gibbs and Gladys Sturdy for measuring the films and preparing the samples, respectively; and Mr. A. Zerwekh for the Plutonium assays.

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RECEIVED MARCH 1, 1958

S-ADENOSYLMETHIONINE AND ERGOSTEROL SYNTHESIS[1]

Sir:

The side-chain C-28 methyl group of ergosterol have been shown to arise intact from methionine.[2,3] This transfer of a methyl group was of interest since this was the first demonstration that a carbon atom may act as the acceptor of a methyl group. In order to participate in certain methyl additions, methionine must first react with ATP[4] to form an adenosylsulfonium compound, S-adenosylmethionine,[5] which may then act as the methyl donor to the appropriate acceptor. Involvement of S-adenosylmethionine as the actual donor in the transfer of methyl groups to sulfur[6] and nitrogen[7] has been de-

(1) Work done under the auspices of the Atomic Energy Commission.

(1) This work was performed under the auspices of the U. S. Atomic Energy Commission.
(2) G. J. Alexander, A. M. Gold and E. Schwenk, THIS JOURNAL, **79**, 2967 (1957).
(3) G. J. Alexander and E. Schwenk, *ibid.*, **79**, 4554 (1957).
(4) H. Borsook and J. W. Dubnoff, *J. Biol. Chem.*, **171**, 363 (1947).
(5) G. L. Cantoni, THIS JOURNAL, **74**, 2942 (1952).
(6) S. K. Shapiro, *Bacteriol. Proc. Am. Bacteriologists*, 116 (1957).
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