The combined crystals were recrystallized from a mixture of ethyl alcohol and di-isopropyl ether. The yield was 1.5 g., m. p. 191–192°, after drying at 100° in a vacuum.

Anal. Calcd. for $C_{21}H_{27}NO_2 \cdot HCl$: C, 69.69; H, 7.80; N, 3.87; Cl, 9.80. Found: C, 69.5, 69.3; H, 7.72, 7.56; N, 3.57; Cl, 9.54.

1-Dimethylamino-3,3-diphenyl-2-methylpropane and Salts.—After the removal of 6-dimethylamino-4,4-diphenyl-5-methyl-3-hexanone (Isoamidone II) oxalate from the material obtained by the hydrolysis of the crude ketimine, there remained a mixture of oxalates. A solution of 30.5 g. of this mixture in 300 ml. of 20% hydrochloric acid was boiled eight hours. It was made alkaline to phenolphthalein with sodium hydroxide and extracted three times with ether. A solution of 30 g. of oxalic acid in alcohol was added to the ether solution. The crystals which separated on cooling were recrystallized three times from a mixture of acetone and alcohol. The yield was 7.1 g., m. p. 138–140°.

Anal. Calcd. for $C_{18}H_{23}N \cdot H_2C_2O_4$: C, 69.97; H, 7.29; N, 4.08; $H_2C_2O_4$, 26.24; neut. equiv., 171.5. Found: C, 70.00; H, 7.40; N, 4.14, 4.06; $H_2C_2O_4$, 25.90; neut. equiv., 169.5.

The picrate was prepared by the action of picric acid on the base in alcohol; m. p. 127–129° after crystallization from alcohol and drying at 100°.

Anal. Calcd. for $C_{18}H_{23}N \cdot C_6H_3N_3O_7$: C, 59.75; H, 5.39; N, 11.62. Found: C, 59.55; H, 5.43; N, 11.58, 11.58.

The base was liberated from the oxalate; b. p. 144–150° under 2 mm.

Anal. Calcd. for $C_{18}H_{23}N$: C, 85.37; H, 9.09; N, 5.53. Found: C, 85.45; H, 9.08; N, 5.54.

For the preparation of the hydrochloride, 2 g. of the base was dissolved in a mixture of 1.7 ml. of 20% hydrochloric acid and 2 ml. of water. The mixture was evaporated to dryness and the residue crystallized from isopropyl alcohol and dried at 100° in vacuum. The yield was 1.86 g., m. p. 181.5–183°.

Anal. Calcd. for $C_{18}H_{23}N \cdot HCl$: C, 74.61; H, 8.29; N, 4.84; Cl, 12.26. Found: C, 74.60; H, 8.24; N, 4.80; Cl, 12.30.

Preparation of 1-Dimethylamino-3,3-diphenyl-2-methylpropane from 4-Dimethylamino-2,2-diphenyl-3-methylbutanenitrile.—To a solution of 20 g. of the nitrile in 500 ml. of isopropyl alcohol, there was added 50 g. of sodium. After the reaction subsided, the mixture was boiled one and a half hours. Water and alcohol were added to destroy the remaining sodium. The mixture was poured into water and extracted with ether. The extract was washed with water. Treatment with oxalic acid in the usual way gave 15 g. of the oxalate, m. p. 134–137°. A portion was converted into the picrate, m. p. 128–130°. There was no depression on mixing with 1-dimethylamino-3,3-diphenyl-2-methylpropane picrate obtained as described above.

Acknowledgment.—We are indebted to Dr. V. B. Fish for the analyses and to Miss Mary L. Evanick for technical assistance.

Summary

1. A series of acids and esters derived from the nitriles obtained as intermediates in the synthesis of the Amidones has been prepared.

2. Two anomalous reactions of these nitriles have been studied.

PHILLIPSBURG, NEW JERSEY RECEIVED APRIL 6, 1948

[CONTRIBUTION FROM THE LOS ALAMOS SCIENTIFIC LABORATORY OF THE UNIVERSITY OF CALIFORNIA, LOS ALAMOS, N. M.]

Utilization of Nicotinic Acid and its Amide by the Human Erythrocyte in Vitro[1]

BY EDGAR LEIFER, JOHN R. HOGNESS, LLOYD J. ROTH AND WRIGHT LANGHAM

Recent investigations by Hoagland, Ward and Shank,[1a] and Handler and Kohn[2] indicate that nicotinic acid is a precursor of coenzyme in the human erythrocyte *in vivo* and *in vitro*, whereas nicotinamide is not. In view of these reports, it was felt that studies of the utilization of nicotinic acid and its amide, using tracer techniques, would be of value. These compounds "labeled" with C^{14} have recently been synthesized.[3a,b] *In vitro* studies of the transport of nicotinic acid, nicotinamide, as well as *p*-aminobenzoic acid (all "labeled" with C^{14} in the carboxyl group) are discussed in this paper. Previous *in vitro* investigations have given inconclusive results. Axelrod, Spies and Elvehjem[4] report that nicotinamide is effective for coenzyme synthesis; Handler and Kohn[2] find that at best the amide is only one-third as potent as nicotinic acid. *In vivo* determinations (mice) will be reported along with gross metabolism studies.

Procedure

Five cc. lots of whole, sterile, heparinized, human venous blood were pipetted into a number of sterile stoppered flasks; 0.5 cc. of C^{14} "labeled" nicotinic acid solution (approximately 3,000 counts/sec./cc., equivalent to 0.05 mg. of nicotinic acid) were added to one third of the flasks; 0.5 cc. of C^{14} "labeled" *p*-aminobenzoic acid solution (approximately 3,000 counts/sec./cc., equivalent to 0.05 mg. of *p*-aminobenzoic acid) were added to the second third; and 0.5 cc. of C^{14} "labeled" nicotinamide (approximately 3,650 counts/sec./cc., equivalent to 0.05 mg. of nicotinamide/cc.) were added to the remaining flasks. The contents of the flasks were thoroughly mixed and the temperature maintained at 37°. The flasks were shaken slowly and continuously throughout the experiment. An atmosphere of 5% carbon dioxide in oxygen was provided to maintain normal *p*H.

At various intervals, 0.5-cc. aliquots were withdrawn from each flask, introduced into centrifuge tubes and centrifuged; 0.05 cc. of the supernatant plasma from each tube was plated on copper discs and counted using a helium–alcohol filled G. M. tube with a thin mica window (1.7 mg./sq. cm.). The remaining plasma was removed and the cells were laked with distilled water up to 0.5 cc.; 0.05 cc. of the laked cells were plated and the radioactivity

(1) This document is based on work performed under Contract Number W-7405-eng-36 for the Atomic Energy Project, and the information covered therein will appear in Division V of the National Nuclear Energy Series (Manhattan Project Technical Section) as part of the contribution of the Los Alamos Laboratory.

(1a) Chas. L. Hoagland, S. M. Ward and R. E. Shank, *J. Biol. Chem.*, **151**, 369 (1943).

(2) Philip Handler and Henry I. Kohn, *ibid.*, **150**, 447 (1943).

(3) (a) Arthur Murray, W. W. Foreman and Wright Langham, THIS JOURNAL, **70**, 1037 (1948); (b) Arthur Murray and Wright Langham, unpublished.

(4) A. E. Axelrod, Tom D. Spies and C. A. Elvehjem, *J. Biol. Chem.*, **138**, 667 (1941).

determined as above. The hematocrit of the whole blood was determined to correct for the amount of dilution of the laked red blood cells.

After forty-eight hours, when the solutions appeared to be at equilibrium (see Figs.), samples of blood from the flasks containing the three radioactive compounds were withdrawn, centrifuged and the supernatant plasma removed; 0.5 cc. of the remaining cells were then washed five times with 10 cc. of normal saline in an effort to wash the radioactivity from the cells. The cells were laked, plated and counted as above.

Since the results obtained indicated that nicotinic acid was preferentially absorbed from the plasma, it was decided to repeat the above experiment with radioactive nicotinic acid, substituting a saline solution containing varying amounts of non-radioactive nicotinic acid, for the plasma. In this manner, the mole fraction of radioactive nicotinic acid was varied in order to determine the maximum uptake of nicotinic acid by the red cell. The saline solutions replaced the plasma quantitatively and contained 0, 0.005, 0.01, 0.1, 0.5 and 1.0 mg. of ordinary nicotinic acid/cc. of saline. The values reported were found to be reproducible within 2 counts/sec.

Discussion of Results

The results obtained are shown graphically. In Figs. 1 through 5, the relative concentrations of radioactivity in plasma (or supernatant saline) and cells are plotted against time. The results show that after twenty-four hours the solutions have reached equilibrium.

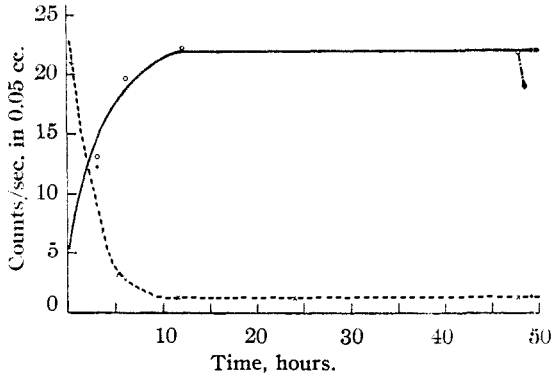

Fig. 1.—Radioactive nicotinic acid in whole blood: ---×---, radioactivity in plasma; —O—, radioactivity in red cells; –·–·–, radioactivity in cells after washing.

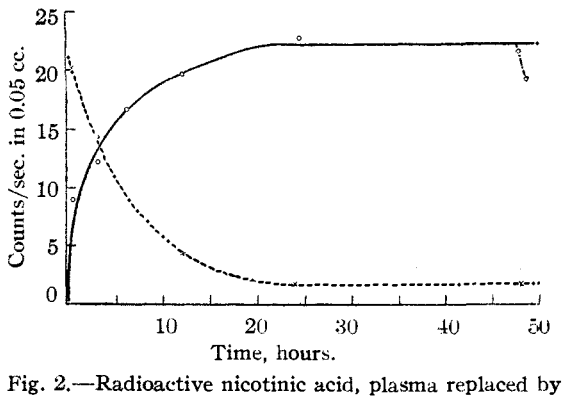

Fig. 2.—Radioactive nicotinic acid, plasma replaced by saline: ---×---, radioactivity in saline; —O—, radioactivity in red cells; –·–·–, radioactivity in cells after washing.

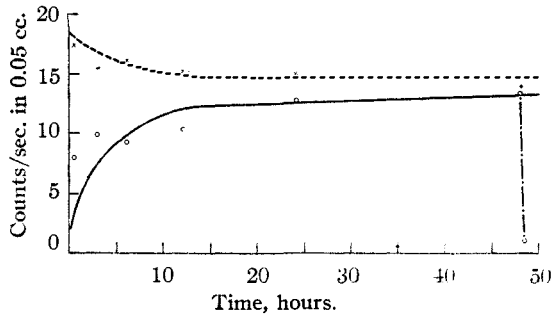

Fig. 3.—Radioactive nicotinic acid, plasma replaced by saline containing 1.0 mg. of nicotinic acid (non-radioactive)/cc. of saline: ---×---, radioactivity in saline; –O–, radioactivity in red cells; –·–·–, radioactivity in cells after washing.

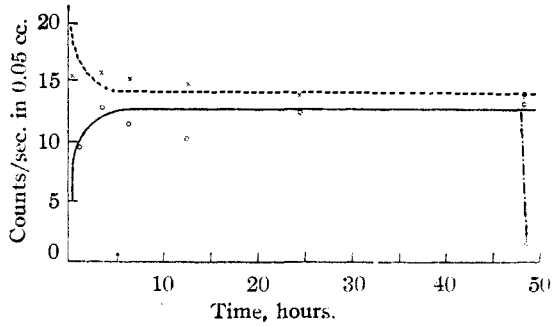

Fig. 4.—Radioactive *p*-aminobenzoic acid in whole blood: ---×---, radioactivity in plasma; –O–, radioactivity in red cells; –·–·–, radioactivity in cells after washing.

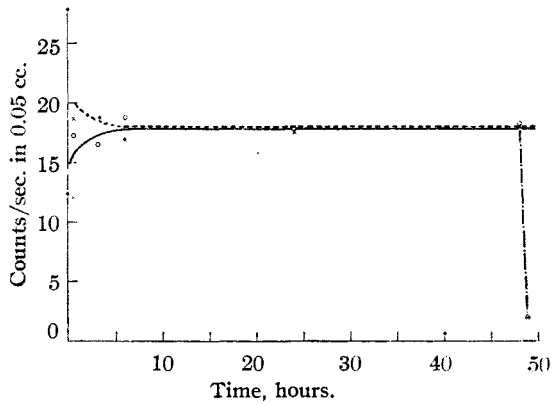

Fig. 5.—Radioactive nicotinamide in whole blood: ---×---, radioactivity in plasma; –O–, radioactivity in red cells; –·–·–·–, radioactivity in cells after washing.

From Figs. 1 and 2 it is seen that nicotinic acid at a concentration of 5 γ/cc. of whole blood is taken up quantitatively by the red blood cells and cannot be completely washed out. On the other hand, as shown in Fig. 4, *p*-aminobenzoic acid is not completely absorbed by the cells and the distribution can be explained by a simple diffusion process. Washing five times with twenty times the volume of normal saline will remove 85% of the *p*-aminobenzoic acid from the cells. Figure 5

shows an effect for nicotinamide similar to that observed for *p*-aminobenzoic acid. Absorption is significantly lower than for corresponding amounts of nicotinic acid. The nicotinamide, like *p*-aminobenzoic acid and unlike nicotinic acid, is almost completely washed from the cells. The remaining amount (less than 0.5 γ/cc.) is presumably fixed and utilized.

Incubation of red blood cells with saline containing varying quantities of non-radioactive nicotinic acid plus a constant amount of radioactive nicotinic acid produces the effects seen in Figs. 2 and 3 and in Table I. The dilution of the isotope changes the uptake of the radioactive nicotinic acid as would be expected if the red cells have a saturation point for nicotinic acid. From Table I, this saturation point is reached at about 10 γ/cc. of packed red cells. (This value has been obtained on the blood of two normal men.) This figure is in agreement with that which is calculated from the values given in the *in vivo* experiments of Hoagland, Ward and Shank,[1] if one assumes that daily ingestion of 20 mg. of nicotinic acid/kg. will produce saturation of the erythrocytes.

TABLE I

UPTAKE OF RADIOACTIVITY BY RED BLOOD CELLS AS A FUNCTION OF MOLE FRACTION[a] OF RADIOACTIVE NICOTINIC ACID

Mole % of radioactive nicotinic acid added	Radioactivity in c./sec./0.05 cc. 20 minutes Supernate	20 minutes Cells	24 hours Supernate	24 hours Cells	24 hours Washed cells[b]
1	17.4	8.0	15.0	12.8	1.2
2	16.0	8.8	14.6	12.2	1.4
10	20.3	8.1	13.2	11.3	2.2
50	19.6	7.1	5.2	13.1	10.1
75	21.0	7.1	2.5	15.9	11.0
100	20.0	9.0	1.6	22.6	19.0

[a] In each experiment, 0.005 mg. of radioactive nicotinic acid was added to 1 cc. of whole blood and the non-radioactive nicotinic acid was varied. [b] One-half cc. of cells washed three times with six times the volume of normal saline.

An experiment similar to the one described above was performed substituting mouse blood for human blood. It was found that in this case both nicotinic acid and nicotinamide are utilized and fixed in the erythrocytes to the same extent. The saturation obtained for a series of six mice was 4 γ/cc. of packed red cells. From this it can be seen that there is a species difference in the utilization of nicotinic acid and nicotinamide. This is significant in evaluating the *in vivo* experiments on mice described elsewhere by the authors.[5]

Conclusions

1. Nicotinic acid *in vitro* is quantitatively taken up by the red blood cells and is fixed in the cells in a non-diffusible form, presumably as coenzyme.

2. Nicotinamide and *p*-aminobenzoic acid, like nicotinic acid, are freely diffusible through the cell membrane, but differ from nicotinic acid in that all but a relatively small amount (less than 0.5 γ/cc. of blood) can be removed from the cells with repeated washings—thus confirming a marked difference of utilization of nicotinic acid and its amide in the human erythrocyte.

3. Isotope dilution techniques indicate that for two normal men approximately 10 γ of nicotinic acid are utilized by 1 cc. of packed erythrocytes.

4. Mouse erythrocytes *in vitro* utilize nicotinic acid and nicotinamide to a similar degree.

(5) Lloyd Roth, Edgar Leifer, John Hogness and Wright Langham, *J. Biol. Chem.*, to be published.

LOS ALAMOS, N. M. RECEIVED APRIL 28, 1948

[CONTRIBUTION FROM THE PURDUE RESEARCH FOUNDATION AND DEPARTMENT OF CHEMISTRY, PURDUE UNIVERSITY]

A Grignard Reagent from 3-Chloro-1,1,1-trifluoropropane

BY E. T. McBEE AND ANTHONY TRUCHAN

Grignard reagents containing fluorine offer possibilities for the preparation of many interesting fluoro compounds. Numerous attempts have been made to prepare Grignard reagents from polyfluoroalkyl chlorides, bromides and iodides but with little success in the past. Henne[1,2] treated several fluorine-containing aliphatic halides with magnesium in ether solution and in each instance either reaction failed to occur or an olefin resulted through the loss of the halogens on adjacent carbon atoms. Thus far, there is no evidence that a Grignard *alpha* to a fluorinated group has been prepared except in one instance,[3] for which the art as yet discloses no corroboration.

It has now been found that $CF_3CH_2CH_2Cl$ reacts in an ether solution with magnesium to form the Grignard reagent, $CF_3CH_2CH_2MgCl$. The success of the reaction is dependent upon anhydrous condition. The $CF_3CH_2CH_2Cl$ may be dried satisfactorily over Drierite or by distillation from phosphorus pentoxide. Analytical grade diethyl ether was used successfully. A crystal of iodine or methyl iodide was employed in the usual way to initiate the reaction. The Grignard re-

(1) A. L. Henne, THIS JOURNAL, **60**, 2275 (1938).
(2) A. L. Henne and A. M. Whaley, *ibid.*, **64**, 1157 (1942).
(3) T. J. Brice, W. H. Pearlson and J. H. Simons, *ibid.*, **68**, 968 (1946).