

indicate time in days, and also the concentrations of the alcohol solutions offered each day. The record does not show the 10-day preliminary period during which

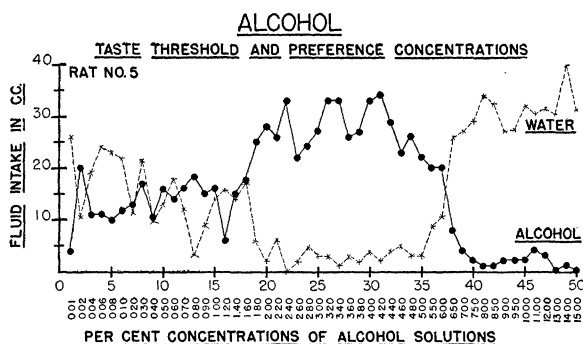


FIG. 1.

both bottles were filled with distilled water. For the first 18 days, when the concentrations increased from 0.01 per cent. to 1.4 per cent., the rat drank almost equal amounts of distilled water and of the alcohol solution. However, it showed a preference for the 1.8 per cent. and for all concentrations up to 4.8 per cent. It drank large amounts of these solutions and small amounts of water. It showed its greatest preference for alcohol solutions from 2.4 per cent. to 4.4 per cent. With higher concentrations its preference decreased. It still showed a slight preference for the 6.0 per cent. alcohol solution, but preferred distilled water to alcohol solutions in all higher concentrations. It drank only minimal amounts of any alcohol solution above 7.0 per cent. Thirteen out of seventeen rats had similar records; three never manifested a preference for alcohol; one preferred water to the alcohol.

The results of some of our previous experiments may throw some light on the significance of these results. Thus far we have found that rats showed preferences for certain concentrations of solutions of substances, such as glucose, maltose, sucrose, galactose, sodium chloride, potassium chloride and dibasic sodium phosphate, all of which are known to play an important part in nutrition. The rats preferred distilled water to poisonous substances, such as mercuric chloride, arsenic trioxide and morphine sulfate, even when offered in extremely low concentrations. These experiments demonstrate that, according to the rats' appetite, alcohol belongs to the group of substances that play a part in normal nutrition.

Further experiments are in progress to determine the effects produced on alcohol taste threshold and maximum preference concentrations by removal of the olfactory bulbs, surgically induced brain lesions, dietary deficiencies, glandular deficiencies and forced alcohol feeding over long periods of time. After long forced alcohol feeding will rats have a higher or lower threshold for alcohol and will they prefer alcohol to

water in concentrations above 6 per cent.? This might give a quantitative measure of any addiction to alcohol.

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THE SYNTHESIS OF NICOTINIC ACID IN THE BODY OF SHEEP¹

ON the basis of growth, evidence² has been presented indicating that nicotinic acid is not a dietary essential for sheep. It has recently been shown that dogs restricted to a typical blacktongue-producing diet after about 28 days virtually cease to excrete nicotinic acid in the urine.³ Rats remain free from deficiency disease when restricted to a diet that is deficient in nicotinic acid only. They continue, however, to excrete appreciable amounts of nicotinic acid in the urine even after being on the deficient diet for long periods of time.³

Since species which require nicotinic acid cease to excrete it when on a deficient diet, its occurrence in the urine of animals restricted to such a diet indicates that it is synthesized in the body. Lambs approximately 3 months of age were placed on a nicotinic acid-deficient diet consisting of regenerated cellulose 20, brewer's rice 49.5, purified casein 9, corn 16, cow peas 2.5, salts mixture 3 and oleum percomorphum weekly to furnish vitamins A and D. Dogs fed this diet, with the modification that the cellulose was omitted, showed a marked decrease in their urinary nicotinic acid and developed typical blacktongue symptoms. After the lambs had been on the experimental diet approximately 8 months the urine was collected for the estimation of nicotinic acid. The nicotinic acid was determined photometrically on unhydrolyzed samples of urine by the cyanogen bromide-aniline reaction.

The figures in the table for the urinary excretion of

Number	Diet	Total per day	Per kg. wt. per day
		mg	mg
418	Deficient	2.52	0.13
420	"	5.33	0.39
488	"	2.81	0.12
443	"	1.95	0.08
443	Deficient + 1 mg n.a./kg wt./day for 13 days	2.86	0.12
443	Deficient + 2 mg n.a./kg wt./day for 5 days	3.00	0.13
443	Deficient + 4 mg n.a./kg wt./day for 13 days	5.16	0.22
3261	Alfalfa hay and grain	2.76	0.09
3439	" " " "	5.74	0.21
523	" " " "	3.23	0.14

¹ Published with the approval of the director of the Texas Agricultural Experiment Station as Technical Contribution No. 592.

² P. B. Pearson, H. Schmidt and A. K. Mackey, *Proc. Soc. Exp. Biol. and Med.*, 40: 423, 1939.

³ L. J. Harris and W. D. Raymond, *Biochem. Jour.*, 33: 2037, 1939.

nicotinic acid by sheep on various regimens are average values for 3 consecutive 24-hour collections. While there is considerable variation in the amount of nicotinic acid excreted in the urine the level is not essentially different, irrespective of whether or not the diet is deficient. Supplementing the deficient diet with nicotinic acid augmented the excretion. The most probable explanation of the continued excretion of nicotinic acid by sheep on a diet deficient in this constituent is that this species can synthesize it either in its tissues or it is formed in the rumen by microorganisms, a process analogous to the synthesis of thiamin, riboflavin, B₆, and pantothenic acid.⁴

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BREAKDOWN OF SULFANILAMIDE MOLECULE BY ULTRA-VIOLET IRRADIATION OR CHEMICAL OXIDATION

In the course of some experiments with ultra-violet irradiation it was observed that dilute aqueous solutions of sulfanilamide became more acid (glass electrode or methyl red as pH indicator). This was taken as an indication of oxidation of the sulfonamide group with liberation of acid sulfur radicals. Proof of a breakdown of the molecule was obtained in that irradiated solutions showed the presence of ammonia by reacting strongly to Nessler's reagent, and of inorganic sulfur by giving a precipitate with barium chloride in acid solution. That the free amino group is also affected has been previously shown by Ottenberg and Fox¹ by a decrease in the diazo reaction under such conditions. By the use of light filters the most effective wave lengths were found to be below 270 mμ, the same region of the spectrum shown by Fox² to bring about the colored products.

The amount of sulfur split off was a function of the length of irradiation. When aqueous solutions of sulfanilamide containing 40 mg per cent. were irradi-

ated by a Hanovia Mercury Arc Lamp at 20 cm distance for 2, 4, 8 and 16 minutes the amounts of sulfur split off were 8.6, 16.1, 29.1 and 40.8 per cent., respectively, of the total amounts present. Varying the concentration of sulfanilamide from 20 to 100 mg per cent. caused only slight variations in the total amounts of inorganic sulfur recovered after a uniform exposure of 10 minutes.

Irradiation of the ortho and meta isomers of sulfanilamide, of acetyl sulfanilamide and of sulfanilic acid for 10 minutes under the above condition did not bring about similar changes, except for the liberation of some ammonia with sulfanilic acid.

Oxidation of sulfanilamide by chemical agents has been carried out by Shaffer³ whose attention was directed to the amino group. Preliminary experiments have shown that the oxidation of dilute aqueous solutions by ferric chloride and hydrogen peroxide is also attended by the liberation of ammonia and inorganic sulfur. The amount of sulfur split off was likewise dependent upon the amount of iron added, indicating that the process is not a catalytic reaction.

After standing at room temperature for 18 hours, 400 cc of an aqueous solution containing 100 mg of sulfanilamide, 2.2 mg FeCl₃ and 0.5 cc 3 per cent. H₂O₂ showed 15.7 per cent. of sulfur split off. Under similar conditions the ortho isomer showed 9.7 per cent., the meta isomer 4.8 per cent. and sulfanilic acid (neutralized) 18.3 per cent. of sulfur split off. These results are not comparable to those with irradiation, which was applied for only 10 minutes.

Because of the irreversible nature of these changes it is obvious that potentiometric studies of such solutions are not valid. Whether or not the body is capable of splitting off any of the sulfonamide group remains to be demonstrated. The demonstration by James⁴ of p-aminophenol in the urine following sulfanilamide therapy is evidence to this effect.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SPEEDIER AND LESS COSTLY METHOD OF CONCENTRATION IN NITRO-CELLULOSE IMBEDDING

In the hot nitrocellulose method of imbedding histological and cytological material as developed by

Jeffrey,¹ and somewhat elaborated by Wetmore,² chips of celloidin in small metal troughs are used to absorb the ether and alcohol and thus concentrate the liquid in which the material is being imbedded. Around any laboratory using this process a certain amount of used celloidin soon accumulates. It often contains particles of imbedded material as well as dirt from containers

⁴ L. W. McElroy and H. Goss, *Jour. Biol. Chem.*, 130: 437, 1939.

¹ R. Ottenberg and C. L. Fox, Jr., *Proc. Soc. Exper. Biol. and Med.*, 38: 479, 1938.

² C. L. Fox, Jr., J. E. Cline and R. Ottenberg, *Jour. Pharm. and Exper. Therap.*, 66: 99, 1939.

³ P. A. Shaffer, *SCIENCE*, 89: 547, 1939.

⁴ G. V. James, *Biochem. Jour.*, 33: 1688, 1939.

¹ E. C. Jeffrey, *Bot. Gaz.*, 86: 456-467.

² R. H. Wetmore, *Stain Tech.*, 7: 37-62.