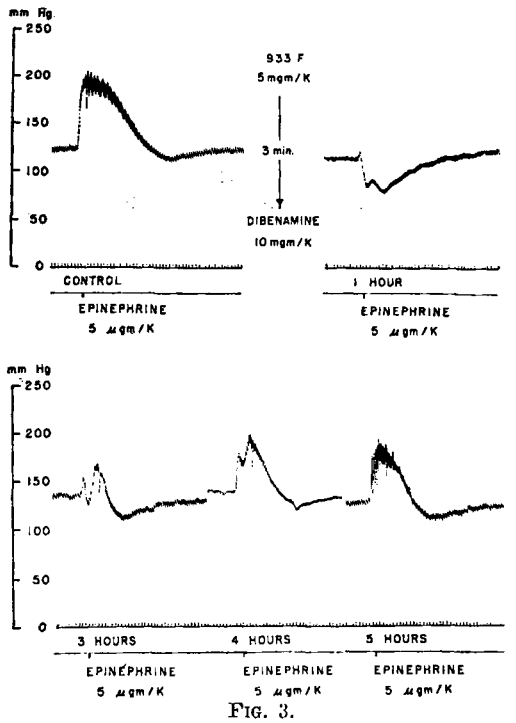

FIG. 3.

Dog, 13.8 kg, female. Sodium pentobarbital anesthesia 30 mg/kg. From top to bottom: carotid blood pressure, 6-second time intervals, injection marker. The 5 blood pressure responses to 5 μg/kg of epinephrine are (reading from left to right): one of 3 control responses obtained before the injection of 933F and Dibenamine, and, respectively, the responses obtained 1, 3, 4, and 5 hours after the injection of 5 mg/kg of 933F followed in 3 minutes by 10 mg/kg of Dibenamine.

pressed only slightly the rate of recovery of the pressor response to epinephrine from that obtained with 933F alone.

Discussion. The fact that 933F prevents Dibenamine from blocking the vasopressor effect of epinephrine may be interpreted in either of two ways. 1. The 933F may aid in the destruction of Dibenamine by either chemically combining with the Dibenamine or enabling something else to do so. 2. The 933F may prevent the Dibenamine from combining with the epinephrine receptors by protecting the receptors. From theoretical considerations and experiments now in progress the latter is considered to be the more likely explanation.

Summary. 5 mg/kg of 933F will prevent 10 mg/kg of Dibenamine from blocking the vasopressor effect of epinephrine in the dog.

The 993F used in these experiments was supplied by Dr. John E. Howard, who obtained it through the courtesy of E. Fourneau.

17047

Contamination of Commercial p-Aminohippuric Acid with p-Aminobenzoic Acid.

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(Introduced by Homer W. Smith.)

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While investigating the oral administration of p-aminohippuric acid (PAH) in man for the purpose of renal clearance measurement, it was noted that different samples of PAH, when administered according to the same routine, gave markedly different concentrations of chromogen in the plasma. PAH was determined by the method of Smith *et al.*[1] in a cadmium sulfate filtrate of plasma.

The plasma concentration of chromogen following the ingestion of 5-6 g of material in divided doses averaged 2.4 mg % (expressed as PAH) in 23 subjects receiving lot No. 12322* while the plasma concentration of chromogen averaged 0.22 mg % in 14 subjects receiving lot No. 236518.* Four tests were then performed on 2 subjects so that

1 Smith, H. W., Finkelstein, N., Aliminosa, L., Crawford, B., and Graber, M., *J. Clin. Invest.*, 1945, **24**, 388.

* National Aniline Division, Allied Chemical and Dye Corporation.

each received each lot of PAH under identical conditions. Lot No. 12322 gave an average plasma concentration of chromogen of 2.76 mg % in one subject and 3.05 mg % in the second, whereas lot No. 236518 gave an average plasma concentration of 0.112 mg % in the first subject and 0.329 in the second. A third subject, started on lot No. 12322 by mouth attained a plasma concentration of 2.4-3.6 mg %. When he was changed to lot No. 236518, the plasma concentration fell to 0.41 mg %. A 50 to 50 mixture of the 2 lots produced a plasma concentration about midway between the figures produced by the compounds when administered separately.

To rule out the possibility that one compound was being metabolized, a number of experiments were performed on dogs, contrasting parenteral with oral administration. The two lots gave essentially the same plasma concentration when administered parenterally. However, a marked difference between them appeared after oral administration.

In identical experiments, lots No. 12322, No. 236518 and p-aminobenzoic acid (PAB) were compared on oral administration. Again the two lots of PAH produced a marked difference in plasma chromogen. In the dog receiving an equivalent dose of PAB, plasma chromogen concentration successively increased from 3.18 mg % at 10 minutes to 14.6 mg % at 51 minutes, with a slow decline apparent after the latter time. The plasma chromogen values in this dog were as much as 100 times as great as those produced typically by lot No. 236518.

Chemical studies. Intensity of color development. Lot No. 12322 yielded about 5% greater color per unit weight at a wave length of 540 mμ. Absorption curves from 400 to 600 mμ on a 0.1 mg % solution of each lot were identical except for the difference in intensity noted above.

Titration curve. 10 cc aliquots of 100 mg % solution prepared with carbon dioxide-free water were titrated with 0.0107 N barium hydroxide solution and the hydrogen ion concentration determined with the Cambridge pH meter. Lot No. 12322 had about 5% more available acid than lot No. 236518.

Solubility curves. Increasing quantities of each lot were placed in tubes with a measured amount of water and shaken in a constant temperature water bath at 24.6°C until equilibrium was attained. The concentration of acid in the liquid was then determined by titration with barium hydroxide. Lot No. 12322 showed increasing solubility with increasing quantities of excess substance, indicating the presence of a mixture, whereas lot No. 236518 showed a sharply breaking solubility curve with constant solubility in the presence of excess substance, indicating a relatively pure compound.

By methods of approximation it was estimated that impurities present in lot No. 12322 comprised between 17 and 31% by weight.

Infra-red absorption spectra. Infra-red absorption spectra on the above two lots and on a third lot (No. 12738) confidently known to be pure PAH,† showed identical absorption bands with correct characteristics of a p-aminophenyl substitution. M-aminohippuric acid showed a quite different and characteristic absorption spectrum.

The melting point (uncorrected) of lot No. 12322 was 165-169°C, of lot No. 236518, 200-202°C; lot No. 12738, 196-198°C; a 50-50 mixture of lots No. 236518 and 12322 melted at 182-185°C; a mixture of lots No. 236518 and 12738 melted at 186-188°C, and a mixture of lots No. 12322 and 12738 at 159-164°C. The recorded melting point for PAH is 198.5°C,[2] and for PAB, 187°C.[3]

Extraction data. 22 g of lot No. 12322 were extracted with ether and yielded 15 g of PAH, 4 g of PAB, and 1 g of unresolved residue. The PAB was identified as such by its melting point (183-185°C) and the fact that the melting point was not depressed by mixture with authentic PAB. The nitrogen content was found to be 10.2-10.4°C (theoretical for PAB is 10.4). The acid equivalent was 135 (theoretical 137). The preparation

† Kindly supplied to us by the National Aniline Division, Allied Chemical and Dye Corporation.

2 Cohen, P. P., and McGilvery, R. W., *J. Biol. Chem.*, 1946, **166**, 261.

3 Beilstein, *Handbuch der Organischen Chemie*, 1931, **14**, 419.

had the typical crystalline form of PAB.

The PAH extracted above melted at 198-200°C, contained 14.1-14.4% nitrogen (theoretical is 14.4), and had an acid equivalent weight of 189-195 (theoretical is 194.2).

Purification of an aqueous iso-electric solution can be effected by extracting with ether and evaporating the ether to dryness. The residue *dissolved in hot water* yields crystalline PAB on cooling. The ether-extracted aqueous solution can be crystallized directly to give PAH which may be recrystallized from absolute alcohol.

Discussion. We conclude from the above data that lot No. 236518 is essentially pure PAH, whereas lot No. 12322 contains approximately 23% of PAB. Incidentally, it is demonstrated that PAB is rapidly absorbed from the intestinal tract from dog and man, whereas PAH is slowly and poorly absorbed.

PAH is made commercially by the conjugation of p-nitrobenzoyl chloride with glycine, the nitro group being subsequently reduced. Failure to effect complete conjugation, or hydrolysis during reduction, will yield PAB. Since the clearance of PAB is less than the creatinine clearance in dogs,[1] the presence of a significant quantity of this substance in PAH would introduce a large error into the determinations of the renal plasma flow. The Medical Division of Sharp and Dohme, who prepared ampouled PAH for clinical investigation, report that lot No. 12322, which we find to be contaminated with PAB, has never been marketed, and they are now examining all samples for significant contamination by PAB.

Summary. P-aminobenzoic acid is rapidly absorbed from the gastrointestinal tract in dog and man while p-aminohippuric acid is poorly absorbed.

One commercial lot (National Aniline No. 12322) of p-aminohippuric acid was found to be contaminated with 23% of p-aminobenzoic acid.

None of the contaminated material, to our knowledge, has been marketed in ampouled form for clinical investigation and an adequate control is now being maintained on the purity of clinical material.

We are indebted to Joanne Baker Schreiner for technical assistance and to Dr. Conrad Dobriner of the Sloan-Kettering Institute, New York City, for determining the infra-red absorption spectrum of the several compounds studied.

We are also indebted to Dr. R. Keith Cannan for his assistance in chemical purification, and to Sharp and Dohme for parallel chemical studies and for rechecking their file samples of their ampouled material.

17048 P

Recovery of Isotopic Succinate from Urine of Rats Administered Isotopic Acetate.

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To demonstrate that a given pathway is involved in the metabolism of an administered isotopic compound, it would appear essential to determine the isotopic composition of the assumed intermediates in the pathway. Evidence is accumulating that the Krebs or tricarboxylic acid cycle, developed mainly on the basis of *in vitro* evidence, actually operates in the intact animal, and that this scheme participates in the metabolism of acetate.[1-4] In the present investigation it was found that after the administration of isotopic acetate

[1] Krebs, H. A., and Johnson, W. A., *Biochem. J.*, 1938, **32**, 113.

[2] Lorber, V., Lifson, N., and Wood, H. G., *J. Biol. Chem.*, 1945, **161**, 411.

[3] Lifson, N., Lorber, V., Sakami, W., and Wood, H. G., *J. Biol. Chem.*, 1948, **176**, 1263.

[4] Rittenberg, D., and Bloch, K., *J. Biol. Chem.*, 1945, **157**, 749.