magnetically, into which 0·025 N formic acid was introduced dropwise in a continuous manner. The eluate was collected in 1-ml fractions; yield, 610 μg, specific activity, 92 μc/μmole.

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A single extraction method for the determination of both norepinephrine and serotonin in brain

(*Received* 23 *January* 1961)

IN THE course of studies in this laboratory on the physiologic role of brain norepinephrine and serotonin and the effects of various drugs on their levels, separate extraction methods for the two amines were developed.[1, 2, 3] In these procedures the amines are extracted from brain homogenates into *n*-butanol: serotonin from an alkaline homogenate, and norepinephrine (because of its instability in alkali) from an acid homogenate. The discovery that serotonin can also be extracted into *n*-butanol from an acid homogenate has made it possible to use the unmodified norepinephrine extraction procedure of Shore and Olin[2] for the extraction of both amines.

With the single extraction procedure, it is possible to measure the amounts of each brain amine in separate aliquots of the final acid extract. Neither amine interferes with the determination of the

TABLE 1. RECOVERY OF SEROTONIN ADDED TO ALIQUOTS OF BRAIN HOMOGENATE

Endogenous serotonin	Serotonin added	Total serotonin		Recovery of total serotonin
		Calculated	Found	
μg	μg	μg	μg	per cent
1·60	0·80	2·40	2·45	102
1·72	0·80	2·52	2·47	98
1·34	0·80	2·14	2·01	94
1·92	0·80	2·72	2·67	98
1·60	0·80	2·40	2·28	95

other. Norepinephrine was determined by oxidation, at pH 5, to a highly fluorescent trihydroxyindole[2] and serotonin by its native fluorescence in 3 N hydrochloric acid.[1]

When one volume of salt-saturated 0·01 N hydrochloric acid containing serotonin is shaken with ten volumes of *n*-butanol, about 85 per cent of the serotonin is extracted into the organic phase. Serotonin added to aliquots of rabbit brain homogenates was almost completely recovered (Table 1), and the recovery of norepinephrine added to the same aliquots was the same as previously reported.[2]

The amount of serotonin found in rabbit brainstem by this technique is almost identical with that obtained by the use of the alkaline extraction procedure of Bogdanski *et al.*[1] The single extraction method yielded a mean serotonin content for normal rabbit brainstem of 0·69 μg/g ± 0·05 (s.d.), as compared with 0·66 μg/g ± 0·03 (s.d.), with the alkaline extraction procedure. The serotonin and

norepinephrine levels in the brainstems of cat and dog obtained with the single extraction method were also comparable to those found when the separate extraction procedures were used. Tryptamine, metanephrine, normetanephrine and 3,4-dihydroxybenzylamine, which are also extracted by *n*-butanol from a salt-saturated acid solution, do not interfere with the determination of norepinephrine or serotonin.

5-Hydroxytryptophan (5HTP), which has fluorescence characteristics similar to those of serotonin, is partially extracted at the acid pH level. Usually this does not create a problem because 5HTP is not present in detectable amounts in tissues. However, on administration of 5HTP to raise the tissue level of serotonin, it is advisable to analyze the brain for serotonin by the method of Bogdanski *et al.*, which incorporates a wash to remove the 5HTP.

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Inhibition of the enzymic oxidation of some dihydropyridines by polycyclic aromatic hydrocarbons

(*Received* 13 *January* 1961)

A SOLUBLE flavoprotein that catalyzes the oxidation of reduced ribosyl nicotinamide (NRH) by vitamin K_3 was recently isolated from rat kidney and seminal vesicle.[1] This enzyme also catalyzes the oxidation of certain N^1-alkyl derivatives of dihydronicotinamide, but it is completely inert toward the reduced forms of triphosphopyridine nucleotide, diphosphopyridine nucleotide, and nicotinamide mononucleotide (TPNH, DPNH and NMNH, respectively). The NRH-oxidizing enzyme can be separated from other soluble proteins which oxidize either TPNH or DPNH, or both, in the presence of vitamin K_3 and other quinones or dyes,[2, 3, 4] and does not seem to be identical with any flavoprotein previously described. We have recently observed that the enzymatic oxidation of N^1-(*n*-propyl) dihydronicotinamide is inhibited by very low concentrations ($< 10^{-8}$ M) of benz(*a*)anthracene and some derivatives of this hydrocarbon, as shown in Table 1. The oxidation of NRH was also inhibited by these hydrocarbons to about the same extent. On the contrary, the oxidation of DPNH by vitamin K_3, catalyzed by a kidney enzyme with properties very similar to the flavoprotein described by Märki and Martius[2], was quite unaffected by benzo(*a*)pyrene and 7,12-dimethylbenz(*a*)anthracene at a concentration of 10^{-7} M. Experiments carried out under different degrees of illumination suggested that light was not necessary for the inhibition of the NRH-oxidizing enzyme by benzo(*a*)pyrene. Analysis of the inhibition by the latter aromatic hydrocarbon, according to the method of Lineweaver and Burk[5], showed that it was not competitive with respect to vitamin K_3.

It was also observed that those polycyclic aromatic hydrocarbons that inhibited the NRH-oxidizing enzyme formed red-colored 1:1 molecular complexes with naphthoquinones such as vitamins K_1, $K_{2(5)}$, $K_{2(10)}$ and K_3. Spectrophotometric studies of equimolar solutions (10^{-2} M in methylene chloride) of these benz(*a*)anthracene derivatives and vitamin K_3 revealed that the complex formation was accompanied by the appearance of broad, unstructured absorption bands with maxima in the