obtained in much better yield, by working up the lipids obtained by chloroform–methanol extraction of acetone-pretreated brain tissue. The Folch I fraction[7] prepared from such an extract is run through an Amberlite IRC 120 column which removes cations and most of the contaminating inorganic phosphate. The lipid is then distributed in a biphasic system formed from chloroform, ether, ethanol and water. The product, thus separated from contaminating phosphatidyl serine and monophosphoinositide, is insoluble in methanol. Analysis indicates the simplest composition as $(\text{glycerol})_1$-$(\text{inositol})_1$ $(\text{phosphate})_3(\text{acyl ester})_{2?}$. On brief acid hydrolysis, mono- and diglycerides can be isolated from the hydrolysate. The phosphorus-containing hydrolysis products are very similar to those from triphosphoinositide B except that more inorganic P and less inositol triphosphate are formed. After mild alkaline hydrolysis[6] the predominant phosphorus-containing product analyses as $(\text{glycerol})_1(\text{inositol})_1(\text{phosphate})_3$.

By means of alumina and silicic acid columns[8,9], monophosphoinositide giving the correct analysis has also been obtained from brain lipid extracts. This confirms the recent evidence, obtained by chromatography, for the existence of this lipid in brain tissue[2,6,9].

The present results indicate that as well as monophosphoinositide, brain tissue contains two triphosphoinositides which are probably very closely related in structure, and which are tightly attached to brain proteins. No information has yet been obtained regarding the relation of these to diphosphoinositide or the relative amounts of the various inositol-containing lipids.

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[1] J. FOLCH, *J. Biol. Chem.*, 177 (1949) 505.
[2] L. HORHAMMER, H. WAGNER AND J. HOLZL, *Biochem. Z.*, 332 (1960) 269.
[3] J. FOLCH, *Phosphorus metabolism, Symp.*, Johns Hopkins Press, Baltimore, 2 (1952) 186.
[4] J. FOLCH AND F. N. LEBARON, *Federation Proc.*, 12 (1953) 203.
[5] F. N. LEBARON AND J. FOLCH, *J. Neurochem.*, 1 (1956) 101.
[6] R. M. C. DAWSON, *Biochem. J.*, 75 (1960) 45.
[7] J. FOLCH, *J. Biol. Chem.*, 146 (1942) 35.
[8] R. M. C. DAWSON, *Biochem. J.*, 70 (1958) 559.
[9] D. J. HANAHAN, J. C. DITTMER AND E. WARASHINA, *J. Biol. Chem.*, 228 (1957) 685.
[10] L. E. HOKIN AND M. R. HOKIN, *J. Biol. Chem.*, 233 (1958) 800, 818.

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A mechanism of *ortho*-hydroxylation of aromatic amines *in vivo*

Several groups[1–3] have obtained data with various species which suggest that separate enzyme systems catalyze the *p*-hydroxylation and *o*-hydroxylation of aromatic amines and amides *in vivo*. It was recently found in this laboratory that N-hydroxy-

2-acetylaminofluorene is a major metabolite in the rat of the carcinogen 2-acetylaminofluorene[4]. Subsequent studies[5] on the metabolic fate of this new metabolite have shown that while it yields the same urinary metabolites given by 2-acetylaminofluorene[6] it gives rise to 3 to 4 times as much 1-hydroxy-2-acetylaminofluorene and less of the other phenolic metabolites than does the parent amide. This suggested that the N-hydroxy derivative may rearrange *in vivo* to form the 1-hydroxy derivative, possibly in a manner analogous to the rearrangement of arylhydroxylamines to phenolic amines in acid[7]. Data in support of this hypothesis are presented herein.

Adult male albino rats, 8-weeks old, were fed 0.03 % of 2-acetylaminofluorene in a purified diet for 6 weeks to produce the liver changes which insure the excretion of the N-hydroxy derivative when the parent amide is fed[5]. The rats were then fed the purified diet alone for 6 days to permit metabolites of the amide to disappear from the urine. Each rat was fed a mixture (equimolar) of 0.97 mg [9-^{14}C]-2-acetylaminofluorene ($2 \cdot 10^6$ counts/min/μmole) and 1.04 mg of unlabeled N-hydroxy-2-acetylaminofluorene in 1 g diet. The urine was collected for the next 6 h. Following the procedures of WEISBURGER *et al.*[8] the urinary metabolites were released enzymically from their conjugates, extracted with ether, and separated chromatographically on a silicic acid column with cyclohexane–*tert.*-butanol–acetic acid–water (18:2:2:1, v/v/v/v). This yielded satisfactory samples of the N-, 5-, and 7-hydroxy derivatives of 2-acetylaminofluorene. A band containing a mixture of the N-, 1-, and 3-hydroxy derivatives was resolved by descension chromatography on Whatman No. 1 paper with the above solvent mixture after the paper had been thoroughly extracted with this solvent mixture. The compounds were eluted from the paper with ethanol. The absorbancy ratios of the spectra of the various eluates were in good agreement with the corresponding values for the authentic derivatives measured under the same conditions. The eluates were plated onto aluminum planchets from ammoniacal ethanol solutions and the radioactivities were determined in a gas-flow end-window Geiger counter. As shown in Table I the 1-hydroxy derivative had only about one-fourth the specific activity of the other phenolic metabolites. The specific activities of the urinary 2-acetylaminofluorene and N-hydroxy-2-acetylaminofluorene are consistent with the fact that these compounds are interconvertible to some extent *in vivo*[5]. Previous work[5] has also shown that the precursor of all the hydroxy derivatives is a compound which retains the acetyl group of the administered parent amide.

TABLE I

DISTRIBUTION OF RADIOACTIVITY IN URINARY METABOLITES AFTER ADMINISTRATION OF A MIXTURE OF [9^{14}C]-2-ACETYLAMINOFLUORENE AND UNLABELED N-HYDROXY-2-ACETYLAMINOFLUORENE TO RATS

Specific activity of administered [9^{14}C]-2-acetylaminofluorene = $2 \cdot 10^6$ counts/min/μmole.

Urinary metabolite	*Specific activity (counts/min/μmole)*
2-Acetylaminofluorene	$0.78 \cdot 10^6$
N-hydroxy-2-acetylaminofluorene	$0.60 \cdot 10^6$
1-hydroxy-2-acetylaminofluorene	$0.40 \cdot 10^6$
3-hydroxy-2-acetylaminofluorene	$1.2 \cdot 10^6$
5-hydroxy-2-acetylaminofluorene	$1.7 \cdot 10^6$
7-hydroxy-2-acetylaminofluorene	$1.8 \cdot 10^6$

These results demonstrate that in the rat the 1-hydroxy metabolite of 2-acetylaminofluorene arises largely, if not entirely, from the N-hydroxy metabolite, whereas the 5- and 7-hydroxy derivatives come largely, if not exclusively, from the parent amide. The intermediate activity of the 3-hydroxy metabolite indicates that some of this *o*-hydroxy derivative is derived from the N-hydroxy metabolite. Thus, while hydroxylation at the *para* site (7-) and certain of the *ortho* sites (3-, 5-) apparently involves a direct attack at these ring positions by activated oxygen[9], hydroxylation of the other *ortho* site (1-) apparently involves the intermediary formation and rearrangement of the N-hydroxy derivative, probably via the corresponding *o*-quinolimide (cf. ref. 5).

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[1] D. V. PARKE AND R. T. WILLIAMS, *Biochem. J.*, 63 (1956) 12P.
[2] J. H. WEISBURGER E. K. WEISBURGER AND H. P. MORRIS, *Science*, 125 (1957) 503.
[3] R. T. WILLIAMS, *Detoxication Mechanisms*, 2nd Ed., J. Wiley and Sons, New York, 1959, p. 431.
[4] J. W. CRAMER, J. A. MILLER AND E. C. MILLER, *J. Biol. Chem.*, 235 (1960) 885.
[5] J. A. MILLER, J. W. CRAMER AND E. C. MILLER, *Cancer Research*, 20 (1960) in the press.
[6] J. H. WEISBURGER, E. K. WEISBURGER, AND H. P. MORRIS, *J. Natl. Cancer Inst.*, 17 (1956) 345.
[7] H. E. HELLER, E. D. HUGHES AND C. K. INGOLD, *Nature*, 168 (1951) 909.
[8] J. H. WEISBURGER, E. K. WEISBURGER, H. P. MORRIS AND H. A. SOBER, *J. Natl. Cancer Inst.*, 17 (1956) 363.
[9] J. W. CRAMER, J. A. MILLER AND E. C. MILLER, *J. Biol. Chem.*, 235 (1960) 250.

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A specific synthetic polypeptide antigen

All the specific antigens described till now are either natural substances (proteins, polysaccharides) or are derived from them by chemical modification. The large number of reported failures to detect antibodies against various synthetic homopolymers of α-amino acids suggests that the polymers studied are at the most very weak antigens[1]. Out of tens of synthetic polypeptides investigated by STAHMANN *et al.*[2,3] only one particular sample of poly-L-glutamic acid and a multichain copolymer of glutamic acid, leucine, glycine and lysine were reported to be antigenic. The antibodies formed did not precipitate with the homologous synthetic polypeptides, but cross-reacted with related polypeptidyl albumins as well as with various unrelated proteins. The synthetic polypeptides did not inhibit precipitin reactions between their antisera and proteins. MAURER *et al.*[4] reported recently that antibodies to a linear copolymer of glutamic acid and lysine could be detected by passive cutaneous anaphylaxis, but not by the precipitin reaction. Only tobacco-mosaic-virus protein inhibited the anaphylaxis reaction.