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Short Communications

On the acylation of the carcinogen 2-aminofluorene by rat liver *in vitro**

A previous investigation disclosed that rat liver *in vitro* acetylated and deacylated the carcinogen 2-aminofluorene (AF) repeatedly in a cyclic manner[1]. Conceivably, other intermediates of the citric acid cycle or of fatty acid synthesis could also serve as acyl group donors for aromatic amines such as AF. JOHNSON AND QUASTEL[2] have indicated that in the system, pigeon liver extract-rat brain homogenate, the increased rate of conjugation of the aromatic amino group of sulfanilamide with added succinate may be due, in part, to direct acylation by succinate. We have investigated whether this reaction occurs in the liver with AF as the acyl group acceptor. Liver slices were incubated with AF, labeled with carbon-14 in the 9 position, and sodium succinate, and the isotope content of the expected product, N-2-fluorenylsuccinamic acid (SAF), was determined. Analysis by the method of inverse isotope dilution[3] revealed that 0.86% of the available AF-9-^{14}C was acylated to SAF-9-^{14}C (Table I). When AF-9-^{14}C and sodium succinate were incubated under similar conditions, but in the absence of liver slices, 0.89% of the radioactivity appeared as SAF-9-^{14}C. Thus, in contrast to acetate utilization in the acetylation of AF[1], rat liver appears to be incapable of utilizing succinate for *direct* enzymic succinoylation. Likewise, butyrate is not directly transferable to AF since incubation of liver slices with AF-9-^{14}C and sodium butyrate did not yield the acylated derivative, N-2-fluorenylbutyramide, as determined by the unequivocal carrier technique (Table I). As judged from these slice experiments, rat liver acetyl kinase exhibits a high degree of acyl group specificity which is quite similar to that of the purified acetyl kinase of pigeon liver[4].

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TABLE I

ISOTOPIC CARBON CONTENTS OF SAF AND BuAF ISOLATED FROM INCUBATION MIXTURES

Incubation systems (per flask)	*No. of flasks**	*Carrier (200 mg)*	*Specific activity of purified carrier (c.p.m./mg)*	*Calculated acylation** (%)*
Liver slices (1.0 g), AF-9-^{14}C (1.78 μmoles; $6.36 \cdot 10^5$ c.p.m.) sodium succinate (41 μmoles)	6	SAF	164	0.86
AF-9-^{14}C (3.36 μmoles; $1.18 \cdot 10^6$ c.p.m.) sodium succinate (41 μmoles)	1	SAF	52.7	0.89
Liver slices (1.0 g), AF-9-^{14}C (1.84 μmoles; $4.91 \cdot 10^5$ c.p.m.) sodium butyrate (40 μmoles)	4	BuAF	25.6	0.26
Liver slices (1.0 g), AF-9-^{14}C (1.16 μmoles; $4.75 \cdot 10^4$ c.p.m.)	5	BuAF	7.5	$<$ 0.10

* When more than one incubation flask was used, the carrier was added to the combined contents of the flasks.

** Calculated acylation: $\frac{\text{(specific activity of purified carrier) (wt. of carrier) (100)}}{\text{(total substrate radioactivity)}}$

Experimental

SAF was prepared according to the method of HIRS[5]. Ethyl N-2-fluorenylsuccinamate, the ethyl ester of SAF, has not heretofore been reported. This derivative was prepared by the Fischer esterification of SAF. After recrystallization from benzene-ligroin, the compound melted at 160.5–162.0° (corr.).

Anal. Calculated for $C_{19}H_{19}O_3N$: C, 73.8; H, 6.19
Found: C, 73.8; H, 6.20

The synthesis of AF-9-^{14}C of high specific radioactivity as well as detailed descriptions of typical carrier purification procedures have already been presented[1,6]. Animals, preparation of liver slices, incubation medium, and incubation conditions were essentially the same as previously reported[1].

In the purification of carrier SAF from incubation mixtures, advantage was taken of its alkali solubility due to the presence of a free carboxyl group. Continuous ether extraction of the deproteinized mixtures to which the carrier had been added, at pH 9, or a multiple fractional extraction procedure[7], in which the radioactivity was distributed between 0.10*M* Na_2CO_3 and chloroform, removed the major fraction of unchanged AF-9-^{14}C. The carrier was further purified by recrystallizations to constant specific radioactivity. Conversion to its ethyl ester did not alter the specific radioactivity and constituted proof of radiochemical homogeneity. The purification of carrier BuAF isolated from incubation mixtures required its separation from the contaminating N-acetyl derivative of AF-9-^{14}C, which has nearly identical solubility properties. This was accomplished satisfactorily by column chromatography on activated alumina.

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