Table 1. FORMATION OF THE 8,9-DIHYDRO-8,9-DIHYDROXY METABOLITES OF 7,12-DIMETHYLBENZ[A]ANTHRACENE AND OF ITS MONOHYDROXYMETHYL DERIVATIVES WITH RAT LIVER HOMOGENATES

Group	Age* (days)	Sex	Formation of 8,9-dihydro-8,9-dihydroxy-7,12-dimethylbenz[a]anthracene (c.p.m. × 10^{-3})	Formation of 8,9-dihydro-8,9-dihydroxy derivatives of the monohydroxymethyl compounds (c.p.m. × 10^{-3})
1. Rats from litters weaned to cube diet at 15 days old	25	M	313 ± 2	88 ± 9
		F	265 ± 5	85 ± 4
	35	M	272 ± 6	98 ± 8
		F	205 ± 6	92 ± 6
2. (a) Rats from litters denied access to solid food from 15 days old	25	M	15 ± 2	4 ± 1
		F	37 ± 2	2 ± 1
(b) Rats from same litters weaned to solid cube diet at 25 days old	35	M	265 ± 3	95 ± 5
		F	248 ± 6	55 ± 6
3. (a) Rats from litters fed condensed milk from 15 days old	25	M	9 ± 1	4 ± 1
		F	8 ± 1	3 ± 1
(b) Rats from same litters weaned to solid cube diet at 25 days old	35	M	84 ± 3	17 ± 2
		F	98 ± 9	22 ± 3

*Age of rats when liver homogenates were prepared.

In adult animals, both DMBA and 7-OHM-12-MBA are metabolized by hepatic microsomal hydroxylating systems that are enhanced by pretreatment with some foreign compounds[7]. One interpretation of these results is that the increase in the metabolism of DMBA by liver from 25 day old rats is caused by an induction of microsomal enzymes by dietary components first ingested during weaning. The fact that this increase in DMBA metabolism can be delayed in rats restricted to milk diets supports this view. Further support is provided by the results of recent studies of hexobarbital metabolism in mice where variations with age have been examined[12]. Hexobarbital sleeping time was minimal in 21 day old animals and this was found to coincide with maximum liver metabolism: in some earlier work with this drug these effects were not noted[13]. There is some evidence that, in rats, the toxicity of DMBA increases between 25 and 50 days[1]: this also supports the concept of enhanced microsomal enzyme activity in weanling animals.

The apparently transient nature of the increase in DMBA hydroxylating activity of rat liver at weaning is also of interest in view of the report that the chronic administration of DDT to rats causes only a temporary increase in the aniline hydroxylating capacity of the liver: in these same animals DDT caused a continuous increase in the liver enzymes metabolizing hexobarbital, aminopyrine and *p*-nitrobenzoic acid[14].

The increases in the hepatic metabolism of DMBA in rats and of hexobarbital in mice found to coincide with weaning indicate that the possibility of diet-dependent variations in microsomal enzyme systems should not be overlooked, particularly where experiments cover the normal weaning period for the species used.

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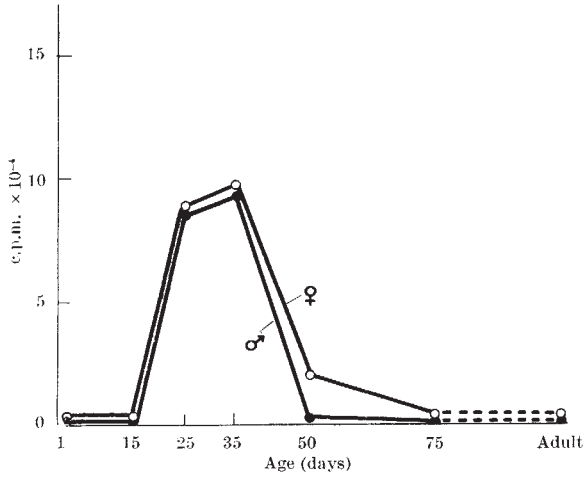

Fig. 2. Formation of the 8,9-dihydro-8,9-dihydroxy derivatives of 7-hydroxy-12-methylbenz[a]anthracene and 12-hydroxy-7-methylbenz-[a]anthracene from DMBA as in Fig. 1.

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[1] Huggins, C., and Morii, S., *J. Exp. Med.*, **114**, 741 (1961).
[2] Morii, S., and Huggins, C., *Endocrinology*, **71**, 972 (1962).
[3] Boyland, E., Sims, P., and Huggins, C., *Nature*, **207**, 816 (1965).
[4] Wheatley, D. N., Hamilton, A. G., Currie, A. R., Boyland, E., and Sims, P., *Nature*, **211**, 1311 (1966).
[5] Huggins, C., Deuel, T. F., and Fukunishi, R., *Biochem. Z.*, **338**, 106 (1963).
[6] Dao, T. L., and Tanaka, Y., *Cancer Res.*, **23**, 1148 (1963).
[7] Boyland, E., and Sims, P., *Biochem. J.*, **104**, 394 (1967).
[8] Wheatley, D. N., Kernohan, J. R., and Currie, A. R., *Nature*, **211**, 387 (1966).
[9] Sims, P., *Biochem. J.*, **98**, 215 (1966).
[10] Huggins, C., and Fukunishi, R., *J. Exp. Med.*, **119**, 923 (1964).
[11] Huggins, C., and Pataki, J., *Proc. US Nat. Acad. Sci.*, **53**, 791 (1965).
[12] Catz, C., and Yaffe, S. J., *J. Pharmacol. Exp. Therap.*, **155**, 152 (1967).
[13] Jondorf, W. R., Maikel, R. P., and Brodie, B. B., *Biochem. Pharmacol.*, **1**, 352 (1958).
[14] Hart, L. G., and Fouts, J. R., *Proc. Soc. Exp. Biol. and Med.*, **114**, 388 (1963).

Multiple Analyses on a Single Gel Electrophoresis Preparation

GEL electrophoresis has been shown to be a rapid means of producing high resolution separations of proteins[1,2], although some of the resolved components may be artefacts when the so-called disc technique is used[3]. It is difficult to correlate the results of replicate disc separations because of variations from one gel cylinder to another[4], but by applying multiple samples to a single acrylamide gel slab[5], using the vertical acrylamide gel slab technique originally described by Raymond[6], individual components of many samples processed together on the same gel slab can be traced across sample boundaries with high reliability, using the principle of continuity[8].

The equipment used is the vertical gel electrophoresis cell of Raymond[6] which provides a gel slab 125 × 175 mm between two parallel water-cooled plates 6 mm apart (Fig. 1). Direct water-cooling of the gel significantly improves the reproducibility from run to run, and makes possible the use of low temperatures (for example, for use with thermally sensitive proteins) or high temperatures (for example, when it is desirable to keep 12 molar urea in solution). The design of the cell makes it unnecessary to rely on gaskets or dialysis membranes to retain the gel during polymerization. The cell is easily dismantled to remove the gel slab at the end of the electrophoresis run. When both running and spacer gels are to be used[1,2], the bottom of the column is occluded by polymerizing a plug of running gel in place, with the cell supported at an angle of 45°. The column is then placed

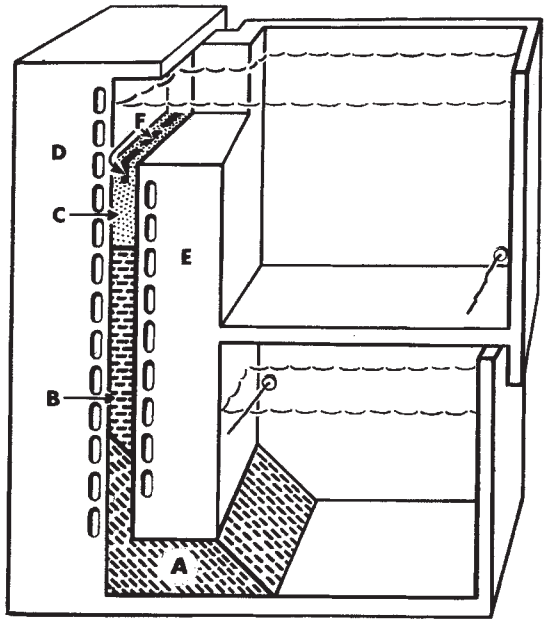


Fig. 1. Vertical gel electrophoresis cell, cross-section showing position of gel slab. *A*, Gel plug; *B*, running gel; *C*, spacer gel; *D*, outer cooling plate; *E*, inner cooling plate; *F*, sample slots.

vertically and filled with running gel solution to the desired level. Water is overlaid to ensure a planar interface between running gel and spacer gel. After polymerization of the running gel, the column is rinsed out and filled with spacer gel solution, which is polymerized in the horizontal position, using a slot-form as described[7]. After polymerization, the gel column is placed vertically, the excess gel and slot form removed, and the electrode chambers filled with buffer. At this point, catalyst residues can be removed from the gel, if necessary, by pre-running with no samples in place. The column is now ready to receive the samples into the sample slots. The use of slots permits up to sixteen samples to be run in parallel under identical conditions without intermixing, and prevents edge effects from affecting the uniformity of the patterns.

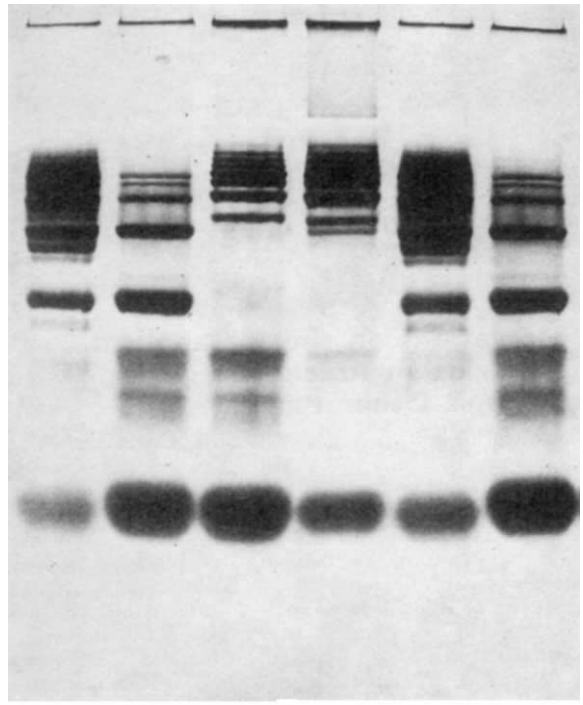
Several points deserve particular mention.

(*a*) The sample is applied in solution, avoiding the artefacts and loss of sample components which occur when the sample is polymerized into a sample gel. The density of the sample solution must be greater than that of the electrode buffer.

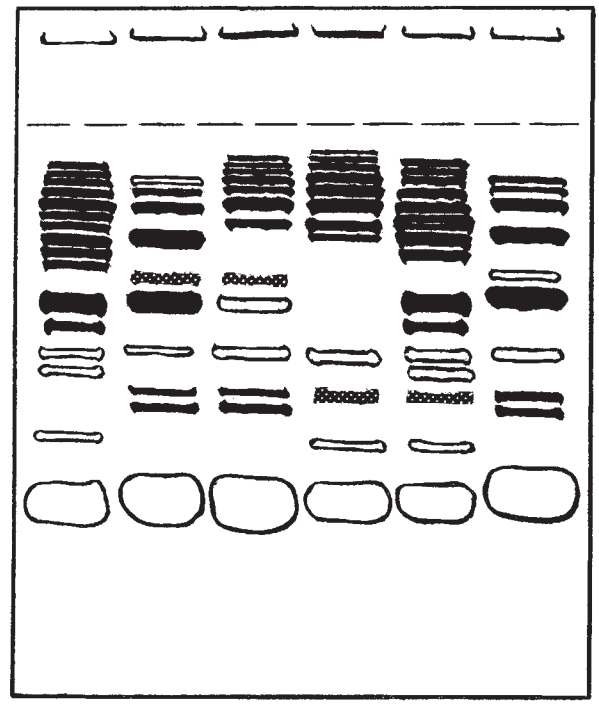
(*b*) In order to maintain a straight buffer front, the sample slots must be filled to the top of the gel with a solution of approximately the same buffer composition and *p*H, and with nearly the same viscosity effect, as the spacer gel. These requirements, and also the density requirement, are conveniently realized by diluting the sample solutions with polymerized acrylamide solution (omitting cross-linking agent) dialysed against the appropriate buffer until free of catalyst residues. A sample is diluted with this buffered acrylamide solution so that a volume sufficient to fill the sample slot contains the appropriate amount of protein. The subsequent electrophoretic separation is performed by the use of standard techniques.

(*c*) In order to preserve direct intercomparability of pattern, especially when individual components are to be identified in several different samples using the principle of continuity, staining techniques must be used which do not require cutting the gel slab apart.

(*d*) For application of different stains, it is usually possible to immerse selected portions of the slab in separate staining solutions without cutting it apart. Alternatively, an agar layer containing the appropriate reagents can be applied to selected portions of the slab. In many systems it is possible to apply different staining solutions in sequence to the entire slab. Fig. 2*A* shows the appearance of one such slab successively stained with



A



B

Fig. 2. *A*, Serum protein patterns, successively stained for lactic dehydrogenase (tetrazolium reaction), haptoglobins (peroxidase reaction), and finally general protein (light green SF). *B*, Diagrammatic representation of *A*. The hatched areas correspond to lactic dehydrogenase, solid black bands to haptoglobins, and outlined areas to material staining only with light green SF.

a lactic dehydrogenase, a peroxidase and a general protein stain.

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[1] Ornstein, L., *Ann. NY Acad. Sci.*, **121**, 321 (1964).
[2] Davis, B. J., *Ann. NY Acad. Sci.*, **121**, 404 (1964).
[3] Brewer, J. M., *Science*, **156**, 256 (1967).
[4] Stuyvesant, V. W., *Nature*, **214**, 405 (1967).
[5] Raymond, S., and Weintraub, L., *Science*, **130**, 711 (1959).
[6] Raymond, S., *Clin. Chem.*, **8**, 455 (1962).
[7] Raymond, S., *Ann. NY Acad. Sci.*, **121**, 350 (1964).
[8] Smithies, O., *Biochem. J.*, **61**, 629 (1955).

Observations on the Metabolism of Fluoride in *Acacia georginae* and Some Other Plants

WHEN working with homogenates of *Acacia georginae* in an attempt to trace the pathway of fluoroacetate synthesis, we found that there was a loss of fluoride, and we extended our experiments to some other plants to study this effect. This communication describes experiments which have led us to believe that plants can convert fluoride, possibly in part, to a volatile form.

A plant of *Acacia georginae*, 15 months old and about 9 in. high, grown from seed, was used. A homogenate was made from 4·6 g of mixed leaves and small roots in the mortar as previously described[1]. The homogenate and washings were reinforced with specially prepared sodium pyruvate (1·0 mmole), manganese chloride (0·1 mmole), potassium dihydrogen phosphate (0·01 mmole brought to *p*H 7·0 with sodium hydroxide), and ATP (0·1 mmole) to make a total volume of 10·8 ml. Three millilitres was kept as a control, and sufficient fluoride was added to the remainder to make a concentration of 40·0 μg/g of plant. The latter sample was then incubated at 30° C with a slow passage of 95 per cent oxygen with 5 per cent carbon dioxide over it for 2 h, after which the two samples were deep frozen. After standing overnight, the homogenates were allowed to thaw and centrifuged at about 50,000*g* for 20 min. The separating solid was washed once with water. Determinations of fluoride were made on both the control and the sample with added fluoride; estimations of inorganic F^- by diffusion and total F^- by diffusion after combustion in the supernatants were made by the technique of Hall[2] and the total fluoride in the "solid" was also determined. The results are given in Table 1.

Table 1. METABOLISM OF FLUORIDE BY A REINFORCED HOMOGENATE OF *A. georginae*

Fluoride added	Fluoride (μg/g wet weight) Inorg. F^-	Total F^-	Solid F^-
Nil	2·4	2·3	2·3
40·0	24·0	24·0	7·2

Loss of fluoride added—33·8 per cent.

Making allowance for the fluoride originally present, the fluoride recovered in the total F^- + solid was 26·5 μg/g, making a loss of fluoride of 33·8 per cent. It is to be noted that this older plant showed no synthesis of organically combined fluoride. Similar and even greater losses of fluoride have been observed in other experiments with homogenates of the Australian plant.

The phenomenon observed with *A. georginae* led to a similar trial with homogenates of other plants taken at random. The results are given in Table 2 and are calculated in the same way as Table 1. Losses of fluoride varied from 15 to 52 per cent.

There seems to be no doubt that the phenomenon is not confined to the *Acacia*, and may be rather general. An attempt to identify the volatile constituent is in progress.

We realize that our conclusions depend entirely on the accuracy of our methods, so we have spent much time in deciding that these losses are not due to experimental error. Errors caused by the fluoride combining with glass were eliminated by using 'Pyrex'. We proved that silicates and various metals combusted with F^- did not interfere and also that our colorimetric stage in Hall's technique[2] was insensitive to acetaldehyde (1·0 mg) and formaldehyde (0·1 mg).

Our final figures are a summation of four separate estimations—that for total fluoride, and for fluoride in the separated solids, except in *Asclepias*, where no solid separated (see footnote to Table 2). Each figure is the average of satisfactory duplicates. Even if we assume that there is an underestimation of 1·0 per cent for the homogenate plus added fluoride and a similar overestimation for the residual fluoride in the control, this would only add up to a total of 8 per cent. Allowing a figure of 5 per cent for possible losses by adsorption on the glass, errors of 15 per cent could be reached by supposing that the errors are all additive. Even so, an experimental error of 15 per cent still leaves a substantial loss in many experiments. We have not yet related the losses to different stages of growth, so the difference between the two experiments on *Asclepias*, taken at different times, is not significant.

We can find no reference in the literature to the conversion of added fluoride to a volatile form by plants, and conclude that the observations are new. We have made some attempt to prove volatility of fluoride by using the radioactive isotope fluoride-18, supplied by the MRC Cyclotron Unit. The short life of this element of about 110 min precludes investigations for longer than 6–7 h. There is also the handicap that we do not yet know the nature of the volatile fluorine compound. Nevertheless, we have passed the mixture of oxygen and carbon dioxide from our homogenates of both *A. georginae* and *Asclepias* successively through water, ethanol and mercury perchlorate, and at the end of 1 and 1·5 h, respectively, a significant amount of radioactivity has been detected in these solutions. The amount is small, but we know from other experiments that, for example, the absorption of methyl fluoride is very inefficient, so that the experiments again show the presence of volatile fluoride.

Our experiments have been made on homogenates *in vitro*, so that it has still to be proved that any effect occurs *in vivo*, or that the losses are all explained by volatility. It is natural to associate the loss of F^- with the formation of ethylene which has been proved to be formed in our homogenates[3]. The aim in our research on the *Acacia* plant has been to find the biochemical path for the synthesis of fluoroacetate. We think it possible that

Table 2. LOSSES OF FLUORIDE FROM HOMOGENATES OF VARIOUS PLANTS

Plant	Part taken	Weight (g)	Volume (ml.)	F^- added (μg/g) wet weight	Per cent fluoride disappearing
Asclepias curassavica	Leaves	3·1	7·0	55·3	31·0
(6 months)	Leaves	2·9	5·3	26·2	52·5
Pea: 'Laxton Superb'					
(1 month)	Seedlings	16·0	12·0	90·5	28·0
Early 'Gradus'	Seeds	12·6	9·0	11·65	26·0
Poa annua	Whole seedling	11·0	8·0	10·42	43·5
	Seedling	5·64	8·0	33·6	14·5
Felicia pappei	Whole	7·0	8·5	15·9	41·5
variety Gracilie	Not in flower	25·8	25·0	40·5	36·0
Aquilegia canadensis	Whole	2·2	5·0	31·8	46·0
(8 weeks)	Whole	3·35	9·0	40·0	15·0
Acacia armata	Leaves and fine roots	3·0	6·2	20·2	33·0
Thea chinensis	New leaves and fine roots	4·3	8·0	35·4	35·0
Doronicum	Flowers	7·6	11·0	35·1	39·5

In the case of *Asclepias*, it was possible to sample the treated homogenate without centrifuging and so to eliminate the extra error due to separate estimation of the solid.