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

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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.

the conversion to a volatile step may be the first stage in the metabolism of fluoride and that it may be a general one for plants. The formation of fluoroacetate would then be caused by a special mechanism in the plants which carry out this step.

If plants in general can contribute to the air around them *in vivo* one or possibly more volatile compounds containing fluorine, it is fortunate that such compounds, though anaesthetic in high concentration, are on the whole non-toxic. This has been proved for gases like vinyl fluoride[4,5].

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IMMUNOLOGY

Increased IgG Synthesis during the Induction of Immunological Paralysis in Adult Guinea-pigs

IT is well known that after immunological stimulation, animals produce non-specific gamma globulins as well as specific antibody[1]. Quantitative studies have shown that injection into guinea-pigs of an antigen mixed with Freund's adjuvant causes a marked increase in the serum concentrations of γ_1 and γ_2 globulins, only part of which can precipitate with the antigen[2]. On the other hand, the induction of immune paralysis in adult guinea-pigs is accompanied by a transient immunological unresponsiveness to unrelated antigens[3]. A possible explanation for this phenomenon could be that immunological paralysis may start by a defective commitment of immunologically competent cells, which does not result in the production of specific antibody. The aim of the present work was to find out whether the induction of paralysis also results in an increase of γ_1 and γ_2 globulins which might account for some kind of commitment of the competent cells.

Random bred adult guinea-pigs weighing 350–500 g were divided into groups and given daily intraperitoneal injections of bovine serum albumin (BSA) according to the following schedules; group 1, 200 μg/day for 12 days; group 2, 100 mg/day for 20 days; and group 3, 1 g/day for 25 days. Blood samples were collected before the beginning of the treatment (day 0) and then at regular intervals. In all groups, including the control group, an immunizing injection of BSA (1 mg) mixed with complete Freund's adjuvant was given to both hind foot pads on day 45. Serum anti-BSA titres were determined by passive haemagglutination using bisdiazotized benzidine BSA conjugated to rabbit red blood cells[4]. The γ_1 and γ_2 globulin content in the serum samples was determined by the antibody–agar plate technique[5] as described before[2]. The results reported are the average values of the analysis of mixed sera (5 to 10) of each group of animals.

In normal adult guinea-pigs the serum content of γ_1 globulin is roughly one-tenth of that of γ_2 globulin, that is 1 mg/ml. and 10 mg/ml., respectively (Fig. 1, day 0). Under the influence of the daily treatment with various amounts of BSA, the serum content of both γ_1 and γ_2 globulins increases progressively until day 30 and then declines. Although the percentage increase of γ_1 globulins is much more important than that of γ_2 globulins, the absolute production of γ_1 is about half that of γ_2 globulins: (1) 2·5 mg/ml. versus 5·5 mg/ml.; (2) 4·5 mg/ml. versus 8 mg/ml.; and (3) 7·5 mg/ml. versus 10·5 mg/ml. in the various schedules of treatment. It is apparent from these figures and from Fig. 1 that the total increase of IgG in the treated animals was proportional to the total quantity of BSA used in the treatment.

Irrespective of the amount of BSA injected, in no case did the treated guinea-pigs produce anti-BSA antibody which could be detected either by Ouchterlony plates or by passive haemagglutination.

On day 45 all the treated animals as well as a control group were again injected with BSA (1 mg) mixed with Freund's complete adjuvant. This injection resulted in a moderate increase of both γ_1 and γ_2 globulins in the control group (Fig. 1*d*), and also in the appearance of high titres of haemagglutinating antibody. This secondary injection of BSA produced much lower passive haemagglutination titres in the animals of groups 1 and 2, showing that a degree of partial paralysis had been induced in these animals by pretreatment with the same antigen. Only in group 3, which had been treated with 1 g of BSA each day for 25 days, was a complete and long lasting state of immune paralysis achieved. In the partially tolerant animals, the challenging injection of BSA produced a fresh increase of γ_1 and γ_2 globulins, comparable with that produced in the control animals. In the completely tolerant animals, however, no such increase was produced and the concentration of γ_1 and γ_2 globulins continued to diminish down to the physiological level (Fig. 1*c*). It seems likely therefore that "non-specific" γ_1 and γ_2 globulin production is a regular sequel of specific antigenic stimulation and is only suppressed if the animal is fully tolerant to that antigen.

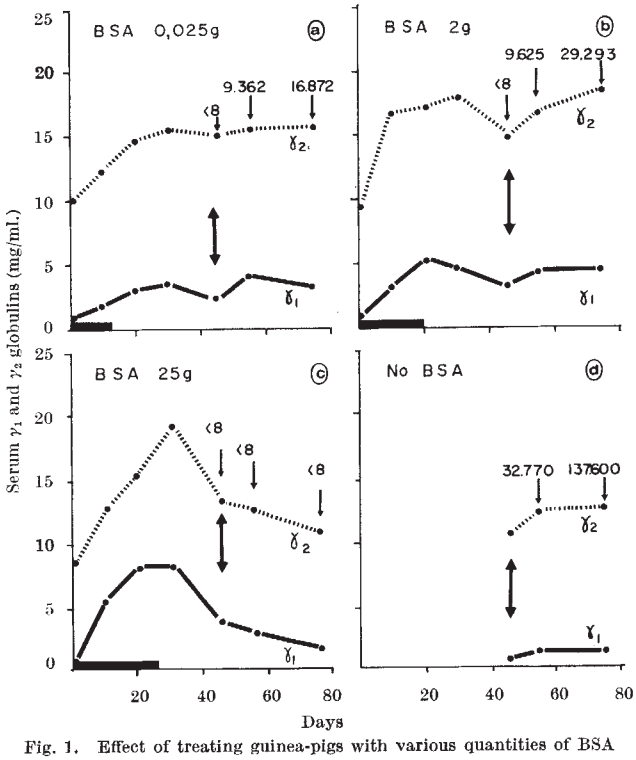

Fig. 1. Effect of treating guinea-pigs with various quantities of BSA on serum γ_1 and γ_2 globulins. ▬, Duration of treatment; ▲, injection of BSA (1 mg) mixed with Freund's adjuvant; ▼, passive anti-BSA haemagglutination titre (for further explanations see text).