Both brochures mention work carried out jointly with the Norwegian Institutt for Atomenergi at Kjeller, near Oslo. About twenty Dutch technicians are working at Kjeller on six different projects, and six Norwegians are assisting with five other joint projects at Petten. At the KEMA laboratories in Arnhem, work on the development of a homogeneous reactor is continuing in collaboration with Euratom. The nuclear fuel for this reactor is to be a suspension of ThO_2–UO_2 particles in water, and the aim is to develop a reactor in which an equal amount of fissile material is produced as that consumed. Methods have been developed for the production of suitable particles—smooth, densely sintered spheres of 2–3μ diameter—and a 250-kW suspension test reactor *KSTR* (*KEMA Suspension Test Reactor*) is being designed. Tests have been made of the damage caused by irradiation of the suspension particles and it has been shown that the degree of damage is strongly dependent on the acidity of the colloid suspension. For these tests new measuring techniques had to be developed, for the investigation consists of colloid chemistry at temperatures above 100° C and represents an entirely new field in the science of chemistry.

S. WEINTROUB

FLUORIDE METABOLISM IN PLANTS

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THE fact that some plants (Chailletaciae) in Africa, especially *Dichapetalum cymosum* (gifblaar) and other species, synthesize fluoroacetate ($F.CH_2.COOH$) raises the general question whether the synthesis of the FC bond is a general property of plants. More recently another plant has been added to the list; the *Acacia georginae* from Central Australia makes fluoroacetate[1,2]. Against the idea that such a synthesis is a general property of plants, there are several known facts.

Among several veterinary investigations, one may quote the recent one made by Shupe *et al.*[3] on cows; the effects of fluoride ingestion led to fluorosis, to change in the bones and teeth. Even so, it took about 4 years for these to develop on diets containing 45 p.p.m., and 2 years on 95 p.p.m. Shupe *et al.* observed no alterations in the soft tissues and no effect on the calves. Such experiments do much to eliminate any latent fear that dosing with small amounts of fluoride may give rise to fluoroacetate. It seemed, however, that there was need for an investigation of the fluoride metabolism of grass, to make sure that it did not synthesize the CF bond. Some preliminary experiments in this direction were made by one of us with Mr. R. J. Hall at Babraham, Cambridge, in which a plot of grass was watered. This was kindly put at our disposal by the director, Dr. I. de Burgh Daly. These did not give decisive results. It was thought that a more direct test on seedlings exposed to varying concentration of fluoride would give more information. These experiments form the basis of this article.

The method published by R. J. Hall[4] was used. In this the diffusion method of Singer and Armstrong[5], somewhat modified, is followed by a modified colorimetric estimation, depending on the formation of an alizarin complexan (Belcher *et al.*[6]). Diffusion is carried out at 60° C from strong perchloric acid. With this method one can detect with reasonable accuracy amounts of fluoride from 0·2 μg to 1·0 μg. 0·1 μg can be detected with errors up to 11 per cent. As devised, the method makes possible the investigation of fluoride metabolism in small samplings of grass seedlings.

Grass seedlings 14–16 days old from Carter's Mixed Seed Invicta with rye grass in lots of 1·0 g (about 20 seedlings) were exposed in beakers for varying periods of time and concentration of fluoride. The 29 experiments done included grass grown on vermiculite, and muslin and 'Terylene' on water. Analyses were made of inorganic fluoride, fluoride acid labile on diffusion, total fluoride by combustion at 600° C, and alkali labile fluoride.

Exps. *a* and *b* in Table 1 show that grass takes up F^- from fluoride solutions. 1*a*, one out of 4 experiments, indicates that this increases from 1 to 4 h exposure at 1·05 mM; 1*b*, that increase of fluoride concentration up to 21·0 mM much increases the uptake. In our early experiments we found 'extra' fluoride by combustion, here called total fluoride, that is, the total F^- was greater than inorganic F^-. We experienced some difficulty in accounting for this, but we now consider that fluoride is also present in the insoluble residue and that differences found between total and inorganic F were due to failure to remove completely the last traces of 'solids' from the supernatants.

Table 1. UPTAKE OF INORGANIC FLUORIDE BY GRASS SEEDLINGS

Exp.	Seedlings grown for:	Conc. F^-	Content of inorganic F (μg/g) at 1·0 h	2·0 h	3·0 h	4·0 h	16·0 h
(*a*)	19 days	1·05 mM	1·5	1·5	1·7	3·4	—
(*b*)	14 days	5·25 mM	28	39	—	30·0	58
		10·50 mM	34	45	—	31	68
		15·75 mM	40	80	—	55	161
		21·00 mM	51	94	—	62	282

1·0 mM = 19 p.p.m.
1·0-g samples (about 20 whole seedlings) were exposed in diffused light to NaF^- in water. After the time stated, the seedlings were thoroughly washed six times quickly with distilled water; excess moisture was removed. They were then weighed and ground in cooled mortar with 1 per cent perchloric acid (2·0 ml.). After centrifuging, the solid was washed three times and supernatants and residue analysed separately.

In support of this, Table 2 gives details of two recent experiments. It shows clearly that when all precautions are taken, even with high concentrations of fluoride representing 300 and 200 p.p.m., no organic fluoride is formed. This view was confirmed by experiments made in the course of the work, where we have not been able to find any extra F^- liberated in this way by alkaline hydrolysis, a characteristic of fluoroacetate. Further, an extract of grass seedlings exposed to fluoride had no fluoroacetate effect on guinea pig kidney homogenate, that is, no extra accumulation of citrate by our usual tests[7].

Table 2. ANALYSIS OF INORGANIC AND TOTAL FLUORIDE TAKEN UP BY GRASS SEEDLINGS FROM HIGH CONCENTRATIONS OF F^-

Seedlings grown for:	Conc. F^- (mM)	Time of exposure	Fluoride (μg/g) supernatant Inorg.	Total	Residue total
11 days	15·75	56 h	1,500	1,515	> 200
17 days	10·50	4 days	448	461	> 40

Details of the experiments were as in Table 1.

In a search through the literature we have only found one instance in which the actual uptake of fluoride has been estimated. Applegate, Adams and Carriker[8] using seedlings of *Phaseolus vulgaris* (minus cotyledons) have determined fluoride taken up at 3 stages of growth in different strengths of fluoride. There was not much difference in uptake between the light and dark. When exposed to 1·0 mM F^- the uptake of inorganic F was 22 μg/g. An increase to 10·0 mM gave an increase of ten times, and with 100 mM the uptake was 2,846 μg/g. In our case a rather steep rise for a long exposure took place between 10·5 and 15·75 mM.

Concentrations of total fluorine may reach 10·0 mM in the soil, but of this only a small amount is present in a

soluble form (personal communication from Mr. L. R. Murray). If all were present as calcium fluoride, the amount which is soluble would be 7·8 μg/g (7·8 p.p.m., or 0·41 mM), well below the amount which gave a significant uptake in our experiments.

We think it significant also that, as our technique improved, the difference between our estimates of inorganic and total fluoride in the extracts made with perchloric acid diminished to zero. We have also no evidence of the presence of organic fluorine in the solid residue. This has been examined in detail in one instance, in the case of grass which had been exposed to 300 μg/g F^- for 3 days. After removal of the supernatant obtained with perchloric acid at room temperature, we subjected the residue to the action of: (*a*) hot perchloric acid, followed by (*b*) extraction with chloroform–methanol solution to extract all lipids, leaving a final insoluble residue: (*a*) contained inorganic F^- 400 μg/g, total F 420 μg/g, a difference within experimental error; (*b*) contained 26 μg/g inorganic F^-. The solid left by this drastic treatment gave a total F^- of 53 μg/g. The presence of fluorine in these extracts and in the final solid raises points of scientific interest; in the present connexion, however, there is no evidence that the material left behind after our first extraction with perchloric acid contained any fluoroacetate. This gives confidence that there is no organic fluoride formed. There is also additional evidence showing the exceptional nature of the capacity to synthesize the organic fluorine compounds. Tea often contains much fluoride; we have analysed a commercial specimen containing 150 μg/g, which did not contain organic fluorine liberated by alkaline hydrolysis. This, is, of course, consistent with the fact that many persons drink large amounts of tea daily without harm. We also watered a *Camellia* plant with fluoride, and found that it took up large amounts of inorganic F^- in the leaves, without forming organic fluoride. In experiments in progress in collaboration with Mr. L. R. Murray on *Acacia georginae*, to which this work is preliminary, the position is different.

Conclusion. Grass seedlings exposed to inorganic fluoride solutions do not take up appreciable amounts of fluoride until concentrations of more than 1·0 mM (19 p.p.m.) are used. No formation of organic fluoride has been found, even with exposure to 15·75 mM fluoride, indicating that there is no formation of fluoroacetate or similar compounds.

We acknowledge help in this preliminary work of Mrs. A. Davies; we also thank Shell Research, Ltd., and the Wellcome Trust for grants for technical assistance, the Wellcome Trust for grants for expenses and Tropical Products Institute for the loan of apparatus. Prof. F. G. Young kindly provided facilities.

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EFFECTS OF WATER POTENTIAL ON GERMINATION OF PEA SEEDS

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WATER shortage can be an important cause of failure of seeds to germinate in the field. This shortage may be due to a high matrix potential[1], or a high osmotic potential, or both, although high osmotic potentials are uncommon under conditions of good husbandry in humid regions. Because of difficulties in producing definable and reproducible conditions at the soil/seed interface, model systems are frequently used in investigations of the effects of moisture on germination, and since matrix and osmotic effects are generally regarded as equivalent in thermodynamic terms, both have been used to produce experimental water potentials.

However, Collis-George and Sands[2] conclude that the results of their work on germination do not support the hypothesis that osmotic and matrix potentials are interchangeable in their quantitative influence on plant behaviour. They consider that the movement of solutes from the soil solution through the cell membranes is a simple diffusion process "because there is not a truly semi-permeable membrane which can permanently exclude the solute external to the cell" and therefore "any detrimental biological consequence is that of an internal *toxicity* rather than an osmotic *drought*" (our italics).

This hypothesis is apparently supported by the results of our own work on the effects of moisture stress as caused by different osmotic potentials on rate and percentage of germination of pea seeds. However, we have evidence suggesting that the testa is in fact differentially permeable and that entry of solute into an intact seed may occur exclusively through the micropyle. At the same time, while working on the effects of different matrix potentials, we have found that the area of contact between pea seeds and liquid water may considerably modify the effect of water potential on germination.

In mannitol solutions of 0, 5, 10 and 15 atm. osmotic potentials (made up according to Morse's modification of the Van't Hoff equation) the germination percentage of 'Kelvedon Wonder' pea seeds was 100, 98·5, 95 and 93 respectively. This was in general agreement with the results of other workers, although the percentage germination in solutions with high osmotic potentials was much higher with pea seeds than others had found with rangegrass[3], alfalfa[4–6], radish[7] and sorghum[7].

Further, the effects of mannitol solutions of 0, 10, 15 and 20 atm. on different parts of pea seeds were traced from a few hours after sowing in Petri dishes until several days after germination (eight observation times). Seeds for sampling were taken from the dishes, and water or

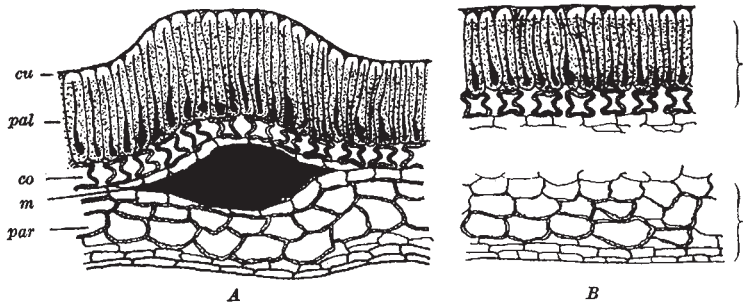

Fig. 1. Transverse section through part of testa of pea seed: *A*, approximate zone of mannitol crystals in parenchyma; *B*, outer (*o*) and inner (*i*) 'parts' obtained by dissection. *cu*, Cuticle; *pal*, palisade; *co*, column cells; *par*, parenchyma; *m*, mannitol