Nutritional Requirements of *C. diphtheriae* and *L. casei*

THE investigations of Lythgoe, Macrae, Todd and others[1] upon rat growth-factors present in liver extracts, and those of Evans, Handley and Happold[2] on the nutrition of *C. diphtheriæ* (types *intermediate* and *gravis*), established the identity of a common growth-factor in these extracts with pantothenic acid. The former investigators also established the presence of another rat growth-factor (factor β) in the amyl alcohol-insoluble fraction of acidified liver concentrates. Active preparations of this factor were found to promote the growth of certain intermediate types of *C. diphtheriæ*, and in 1940 Happold, Lythgoe and Todd commenced a joint investigation with the object of elucidating its nature. The factor could not be extracted by amyl alcohol from aqueous acid solution or by butanol from alkaline solution. At *p*H 1 it was not adsorbed on fullers earth but was readily adsorbed on charcoal (Norite), from which it could be eluted with acetone-ammonia-water. Treatment with 20 per cent hydrochloric or sulphuric acids at 100° for two hours caused little diminution in activity, but similar treatment at *p*H 11 (sodium hydroxide) caused almost complete inactivation. Complete loss of activity was also caused by acetylation, methylation or treatment in the cold with nitrous acid. The growth-factor was not precipitated by phosphotungstic acid, and attempts to purify further via the lead salt were inconclusive, much activity being lost in the process.

More recently, further progress has been made by Chattaway, Happold and Sandford using as test organisms strains of *C. diphtheriæ gravis* of a type at present prevalent in Dundee and obtained through the courtesy of Prof. W. J. Tulloch. The growth-factor for these strains has properties identical with those established for the intermediate type factor, but some additional facts can be given. It is readily adsorbed on Norite at *p*H 3 and 9 but seems less readily adsorbed at *p*H 6·5–7. It cannot be replaced by biotin nor is its activity reduced by avidin, and its properties appear to differentiate it from folic acid; it is insoluble in ethanol and in common organic solvents. The activity of concentrates is largely destroyed on boiling for fifteen minutes with ninhydrin in one third saturated potassium dihydrogen phosphate. It is not extracted by *p*-cresol from acid aqueous solution at *p*H 3; this differentiates it from a growth-factor for *Lactobacillus casei* ε the properties of which had, until this observation was made, appeared to identify it with the *C. diphtheriæ* factor. Another point of difference between the two has been found in the observation that the *L. casei* factor is precipitated as a silver salt at *p*H 7 while the *C. diphtheriæ* factor remains in solution.

It is our intention to publish elsewhere fuller details of the associated studies in our two laboratories, but in view of the general interest in this type of investigation we feel that the position of the work should be indicated at this time.

F. W. CHATTAWAY.
Biochemical Laboratory, F. C. HAPPOLD.
University of Leeds. M. SANDFORD.

Chemistry Department, B. LYTHGOE.
University of Manchester. A. R. TODD.
April 28.

[1] Macrae, Todd, Lythgoe, Work, Hind and El Sadr, *Biochem. J.*, **33**, 1681 (1939); Lythgoe, Macrae, Stanley, Todd and Work, *ibid.*, **34**, 1335 (1940).
[2] Evans, Handley and Happold, *Brit. J. Exp. Path.*, **20**, 396 (1939).

Rumex Lunaria L., a Gynodiœcious Tetraploid Species

THE genus Rumex is divided by modern taxonomists[1] in three sub-genera: Lapathum, Acetosa and Acetosella. In the sub-genus Lapathum only hermaphroditic species occur, but in the other two sub-genera diœcious forms are met. According to studies on the mechanism of sex determination met in these two latter sub-genera, the sub-genus Acetosella belongs to the Melandrium type of localization of sex-determining genes, that is, the *Y* possesses strong male elements, which may dominate over all the female elements in the *X*'s and autosomes in the tetraploid, hexaploid and octoploid species of the subgenus[2,3]. The diœcious species of the sub-genus Acetosa, however, are found to belong to the Drosophila type of localization of sex-determining genes, that is, the *Y*'s are inert, and the sex is determined by a balance between the *X*'s and the autosome sets[4,5,6]. This latter mechanism prevents polyploidy, as shown by Bridges[7], Muller[8] and Ono[4]. The diœcious plants of the sub-genus Acetosa belong to the section Euacetosæ, but in the section Hastati polygamodiœcious and gynodiœcious species are met[3]. Hermaphroditic plants are met in other sections of the sub-genus, namely, Scutati and Vesicarii. Polyploidy has hitherto been known only within the British forms of the collective species *R. scutatus*[9].

According to an explanation given by Löve[3] on the evolution of the two different types of localization of sex-determining genes in the two sub-genera of Rumex, these are to be regarded as two fundamentally different lines of evolution. The type met in sub-genus Acetosella has evolved from hermaphroditic individuals by mutations in the *male* direction followed by chromosomal recombinations over the polygamodiœcious and *androdiœcious* state to the present diœcious state. The type of sub-genus Acetosa has evolved by mutations in the *female* direction from hermaphrodites over polygamodiœcious and *gynodiœcious* plants to the diœcious ones met in section Euacetosæ. According to this hypothesis, the polygamodiœcious and gynodiœcious species of section Hastati should be regarded as a younger state in the same evolution process as the diœcious forms of section Euacetosæ. When the process has evolved to the diœcious state, polyploidy will result in intersexual, sterile forms and in females, as found in the species *R. Acetosa* some few times[4,5,6], but if a polygamodiœcious or gynodiœcious species will become polyploid, no differences in its sex form will, theoretically, be observed. Its way to the diœcious state will, however, be practically blocked.

During the last four years, I have made a number of investigations on the cytogenetics of different species of the genus Rumex. One of the forms examined was the gynodiœcious *Rumex Lunaria* L., which is a Macaronesian species of section Hastati, often cultivated in botanical gardens. My material was obtained from the Botanical Gardens in Lisbon, Portugal, and it is of the same type as herbarium material from the Canaries found in the Botanical Museum at Lund, Sweden.

The only species of the section Hastati hitherto studied, *R. hastatus* D., showed the diploid chromosome number $2n = 18$ [10]. The chromosome number of the species *R. Lunaria* L. was, however, $2n = 36$, or the tetraploid one.

As expected from the above hypothesis, the hermaphrodites of *R. hastatus* and *R. Lunaria*