< ARTICLE >

THE EFFECTS OF UREA NITROGEN ON THE METABOLISM OF PLANTS (II)

The response of some nitrogen components of barley to urea and other nitrogen in water culture.

KIM, Joon Ho

(Dept. of Biology, Kongju Teacher's College)

金俊鎬 : 植物의 代謝에 미치는 尿素窒素의 影響 (II)
大麥根에 吸收된 後의 窒素代謝

(Received April 28, 1962)

ABSTRACT

KIM, Joon Ho (Dept. of Biology, Kongju Teacher's College) : The effects of urea nitrogen on the metabolism of plants. II. The response of some nitrogen components of barley to urea and other nitrogen in water culture. Kor. Jour. Bot. V(2), 6—12. 1962.

For the comparison with the previous paper (4) the present report deals with the absorption and metabolism of urea and other nitrogen ions in barley seedling absorbed through root.

1. The amount of nitrate in barley treated with urea reach it peak on the 8 th day, NO_3 on the 4 th, NH_4 on the 6 th or 8 th, respectively.
2. The ammonia content in urea group reaches its peak on the 6 th day but other groups on the 4 th day. The present data in the urea group show to shorten 4 days compared with that of the previous paper (4).
3. The content of total amide from the present data are gradually increased on all of the groups during this experiment. These are agreement with the result of the previous paper (4).
4. The alcohol soluble nitrogen in the urea group shows the similar tendency to the $NaNO_3$ group but reaches its peak 2 days later than in the $(NH4)_2SO_4$ group.
5. The content of total nitrogen in the urea series has the lowest amount at the beginning while the richest from the 4th day after.

These would be explained on that the absorption of urea is delayed and the PH in the urea solution does not change, so called "physiological neutrality".

The author should like to express his sincere thanks to Prof. M. J. Lee of Seoul National University for his valuable advices.

INTRODUCTION

In the previous paper (4) dealing with the effect of the urea foliar application on the sunflower leaves, author showed that the leaves took very rapidly up the urea through the under surface of them and synthesized the organic nitrogenous compounds with urea. One of the purposes of the present study is to examine the absorption and metabolism when urea is supplied through the root of barley seedling in water culture in comparison with the phenomena in the previous paper. Moreover to compare with the data of urea through the root, the nitrogenous components in the barley seedlings were also determined after supplying nitrate and ammonium salts through the roots.

Oland(6, 7) has broadly made a study of the metabolism of nitrogen after supplying No_3, $(NH_4)_2SO_4$ through root and the urea spray on the leaves. Weissman(22) and Tamm(13) have also reported the results of the absorption and metabolism of nitrate and ammonium salts supplied through root.

As to amide, since Prianichnikov(8, 9) studied, many investigators(5, 8, 18) have indicated that an amide was excessively accumulated with nitrogen supply, but the amount of both asparagin and glutamin were varyed with different sources of nitrogen.

Author herein reports the results obtained from the nitrogens metabolism of barely seedling, when supplied with urea, ammonia and nitrate respectively through root in water culture.

MATERIALS AND METHODS

Material: Seeds of winter barley well sifted were germinated on seed bed which was filled up with moistened fine saw-dust in the greenhouse. When the shoot of seedling attained approximately 10 cm high and all of the embroyonic food might almost be exhausted.

These seedlings were removed, and temporarily planted on the pure water vessel to exhaust the nitrogenous components remained in plant body. These dets were laid down on a stand of 60 cm high in the outdoor for 10 days. Then they were grouped into 4; The urea, $NaNO_3$, $(NH_4)_2SO_4$ and Lack-Nitrogen groups. The first three groups, each containing nitrogen were subdivided into 2 series; the high nitrogen series, containing 120 mg. N per liter, and the low nitrogen series containing 60 mg N per liter. The composition of baisic solution, other than nitrogen element, was as follows; 1.3752 g of $MgSO_4$, 1.5223 g of $CaCl_2$, $2H_2O$, 0.3744 g of K_2SO_4 and 1.149 g of KH_2PO_4 dissolved in per liter, but for the $(NH_4)_2SO_4$ group 0.1878 g of K_2SO_4 and 1.4658 g of K_2HPO_4 instead of 0.3744 g of K_2SO_4 and 1.149 g of KH_2PO_4 dissolved.

As a control, the lack-nitrogen series had only basic elements without nitrogen.

The twenty seedlings of each series were watered in one liter glass bottles respectively. Hydrogen ion concentration in the solutions were not adjusted during this study. The cultivated bottles were arranged according to a Latin square design, furthermore, care was taken to the exposure of the same intensity of light.

Sampling: For the 0 day sample, the plant was picked up before being transplanted in the culture solution, thereafter, samplings picked up at intervals of every two days. The materials were divided into two subsamples: the one was through dried at 70°C. in an oven, weighed, ground and saved for the total nitrogen analysis, the other was directly weighed, ground adding 80 per cent alcohol, and saved in a refigerator below were degree C. till analysed it.

Analysis: The determination of nitrate was according to Schlenker(10). For the other components the analytical methods employed were essentially the same as those of the previous paper (4).

RESULT

Nitrate: The content of nitrate in barley seedlings tended to be rapidly increased from first 4th to 8 th day but decreased thereafter. As will be seen in Fig. 1 the amounts of nitrate in the urea group was increased till the 8th day, the $NaNO_3$ group till the 4th day, the $(NH_4)_2SO_4$ group till the 6th or 8th day, respectively.

It was obvious that the amount of nitrate in the urea group showed to be reached the peak most slowly and also the least in amount compared with other series of both of other groups except the low-$NaNO_3$ series. The differences of nitrate content between the high (120 mg. N) and the low (60 mg. N) series were generally large, though the urea groups did not show so conspicous difference. All of these groups treated with nitrogen equally decreased after 8 th day in this experiment. There was a question; why the nitrate was created in both the urea and the $(NH_4)_2SO_4$ group, in spite of the fact that these was no possibility of their production? The control series kept almost continuously the same amount of nitrate during the experiment.

Ammonia: The content of ammonia was the lowest compared with other nitrogenous components as stated by many workers(2, 5, 9, 15, 16). The amount of ammonia in the urea group had peak on 6th day but

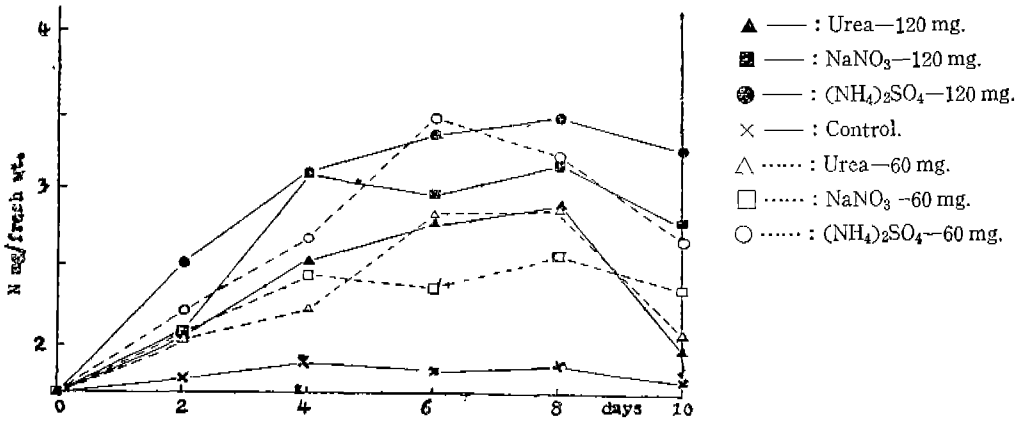

Fig. 1 Effect of urea and some different sources of nitrogen on the content of nitrate in barley seedlings in water culture.
P-values for analysis of variance are : days=39.22**, different source of N=28.09**, amount of supplied N=4.29*, ** Significance of the 1% level. * at the 5% level.

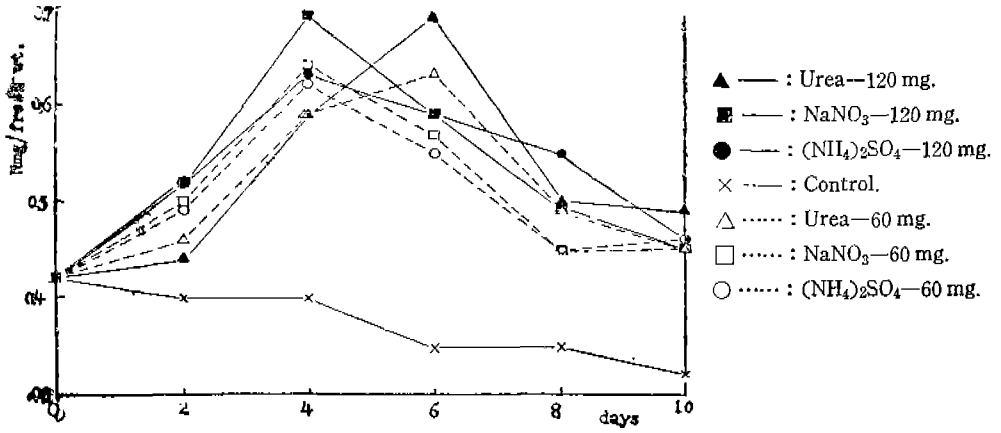

Fig. 2 Effect of urea and some different sources of nitrogen on the content of ammonia of barley seedling in water culture.
P-value for analysis of variance are : days=26.42**, amount of supplied N=12.48. ** and * as in Fig. 1.

both other groups. i.e. $NaNO_3$ and $(NH_4)_2 SO_4$ had on the 4th day, 2 days shortened (Fig. 2). In the nitrogen deficient, control series, it was continuously decreased during this experiment. As shown in Fig. 2, there were no difference of the ammonia content between the low and the high nitrogen series, nor among the different groups.

The result from this study showed the larger amount of ammonia than that by Yemm(18, 19).

Amide: In this study two kinds of amides, i.e. glutamin and asparagin, were determined. The glutamin content was always more than that of asparagin (Table 1). The similar result was obtained by Yemm(18, 19). The amides of both glutamin and asparagin gradually increased by the treatment of the different

Table 1. Effect of urea and some different sources of nitrogen on the glutamin, asparagin and total amide of barley seedling in water culture (N mg g. fresh weight.)

Nitrogen source	mg N	days					
		0	2	4	6	8	10
				Glutamin			
$CO(NH_2)_2$	60	···	1.28	1.28	1.37	1.50	1.91
	120	···	1.38	1.41	1.37	1.61	1.91
$NaNO_3$	60	···	1.17	1.17	1.20	1.32	1.70
	120	···	1.20	1.20	1.25	1.37	1.73
$(NH_4)_2SO_4$	60	···	1.20	1.22	1.27	1.43	1.85
	120	···	1.23	1.27	1.30	1.56	1.81
Lack-N	60	1.20	1.18	1.02	1.09	1.11	1.13
	120						
				Asparagin			
$CO(NH_2)_2$	60	···	0.81	0.97	1.00	0.99	0.95
	120	···	0.90	0.99	0.99	1.03	0.96
$NaNO_3$	60	···	0.70	0.91	0.90	0.99	0.83
	120	···	0.85	0.90	0.96	1.03	0.90
$(NH_4)_2SO_4$	60	···	0.84	0.93	0.96	0.86	0.92
	120	···	0.85	0.93	1.04	0.95	1.00
Lack-N	60	0.51	0.47	0.24	0.13	0.12	0.07
	120						
				Total amide			
$CO(NH_2)_2$	60	···	2.09	2.25	2.37	2.57	2.86
	120	···	2.28	2.34	2.36	2.64	2.87
$NaNO_3$	60	···	1.87	2.08	2.10	2.31	2.53
	120	···	2.05	2.10	2.21	2.40	2.63
$(NH_4)_2SO_4$	60	···	2.04	2.15	2.23	2.29	2.77
	120	···	2.08	2.20	2.36	2.51	2.81
Lack-N	60	1.71	1.65	1.26	1.22	1.23	1.21
	120						

P-values for analysis of variance are : days=25.60**, 1.70, 23.28**, different source of N=10.21*, 73.63**, 58.80**, low and high N content=23.62, 0.19, 0.40, error=40.56, 24.48, 17.52 in glutamin, asparagin and total amide, respectively. ** Significance at the 1% level, * at the 5% level.

sources of nitrogen during this study. The content of amides in the urea groups were more than those of the other groups. The control series was shown to decrease continuously.

Alcohol soluble-N : It is assumed that there are amino acid, amide, polypeptide, chlorophyll and lipids etc. in the 80 per cent alcohol soluble fraction. As will be seen in Fig. 3, the amount of the alcohol soluble nitrogen was increased much on the first 2nd day. The $NaNO_3$ and the urea groups except low series had peaks in the 6th day samples but the $(NH_4)_2SO_4$ group in the 4th day-sample, while decreased after those days. The amount of alcohol soluble nitrogen in the former groups less than that of the letter one. The low-urea series, from the 2nd day, and the control series, from the first, kept the same amount to the end of the experiment. There were neither observed the considerable differnces of the amount of alcohol soluble between the high and the low nitrogen series nor among different sources of nitrogen.

As a whole, in all of the experimented series the content of the alcohol soluble nitrogen shown were rapidly increased with more or less differences till the 2nd day.

Total nitrogen: The content of the total nitrogen were determined only the high nitrogen (120 mg. N) series and were summarized in Fig. 4.

The urea series had, at first, the lowest content compared with both other treated series, while the richest on the 6th day. The $NaNO_3$ series was the highest in the 2nd day but slowly reducing after it. The $(NH_4)_2SO_4$ series was also increased till the 4th day, remained almost that level till at the end of the experiment.

The control series continued to have the same amount during this experiment.

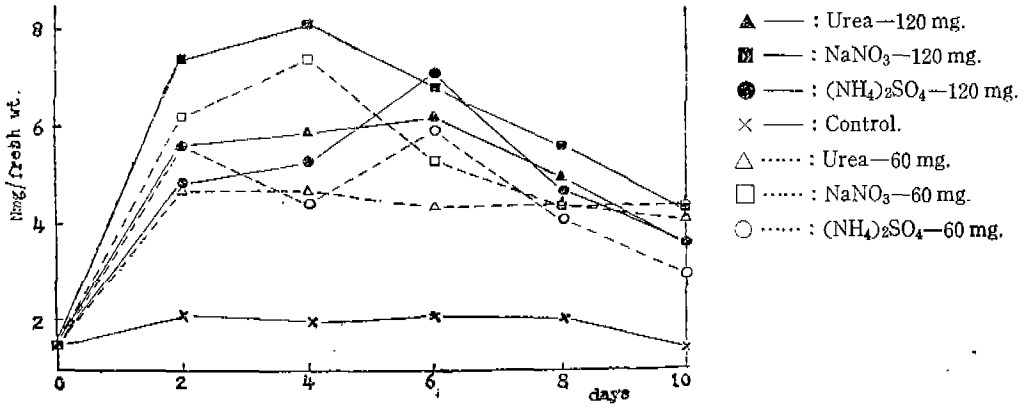

Fig. 3 Effect of urea and some different sources of nitrogen on the content of 80% alcohol soluble *nitrogen of barley seedling in water culture.*

P-values for analysis of variance are : days=45.03**, different source of nitrogen=37.56**, amount of supplied nitrogen=1.48. ** and * as in Fig. 1.

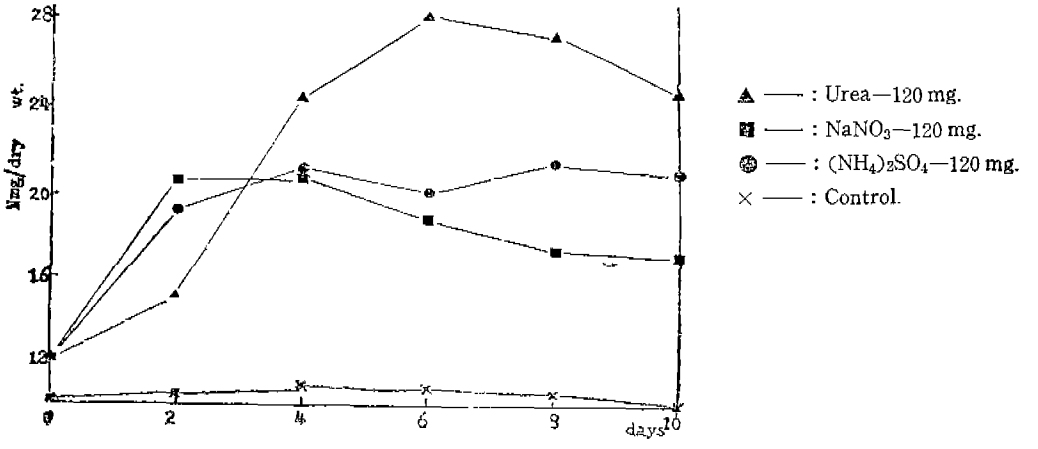

Fig. 4 Effect of urea and some differnt sources of nitrogen on the total nitrogen of barley seedling in water culture.

P-values for analysis of variance are : days=24.97*, different sources of nitrogen=55.03**. ** and * as in Fig. 2.

DISCUSSION

The experiment described here obviously shows that the nitrogen starved barley plant can absorb and utilize urea through the root in water culture, even though it takes up urea more or less slowly compared with both sodium nitrate and ammonium sulfate. In order to evaluate quantitatively the interrelationships among the internal compounds, these data obtained in this experiment were reduced statistically by the analysis of variance. The statistics show that the variation of nitrate in the seven series were highly significant among the samples with the lapse of every two days and the difference of nitrogen sources.

The significant difference was also between the low and the high nitrogen series. The nitrate content in the urea group tend to be poorer than those of the other series of both other groups except low-$NaNO_3$ series; the former reaches peak in the 8th day-sample but both latters reach more or less earlier (Fig. 1). However, it is actually difficult to explain the process of the making of nitrate by urea or ammonium sulfate, because it has been known that urea is hydrolized into ammonia, and then ammonia is assimilated to organic nitrogen. The maximum amount of ammonia in the urea group, as compared with that in the sodium nitrate and ammonium sulfate groups, is synthesized obviously late. The one reaches its maximum content on the 6th day, while the others on the 4th day. According to the previous paper (4) on the study of the urea foliar application to sunflower leaves its maximum content was shown on the 2nd day, 4days shortened.

The question is whether the cause of such difference of time between the present data and that from previous paper (4) is dependent upon the amount of the suplied nitrogen, the assimilation ability from ammonia to other nitrogoenous components or the speed of hydrolysis of urea to ammonia. It may be uncertain to think the first two are due causes because the maximum ammonia content is 0.7 to 0.8 mg. N/fresh weight in both barley seedling and sunflower leaves treated with nitrogen either through root or sprayed on leaves [refer to Fig. 2 in the previous paper (4)]. It would be rather reasonable that this is due to the speed of hydrolysis because barley had scarcely 0.44 to 0.46 mg. N/fresh weight of ammonia on the 2nd day-sample. Therefore the formation of ammonia is quickened about 4 days by the urea foliar spray compared with the absorption through the root.

Early Prianishnikov(8, 9) studying with the seedling of barley and garden pea proved that the amide was increased with the supply of ammonium salts. Mothes(5), who working with the detached leaf of *Phaseolous ultiflorus*. Greenbill and Chibnall(2) with also the detached leaf of *Lolium perennen*, Vickery et al(14) having with sugar beet, obtained similar result. Boynton(1) and Oland(6) with apple leaves have shown that the amide content was enriched with the spray of urea. According to the data from the previous paper (4) the amide was gradually increasee by the urea foliar spray till the 4th day. The data at present also show the increase of amide in the same manner. It is noteworthy that the content of glutamin, asparagin and total amide are generally the most in the urea group. It seems to be reasonable to think amide may be a *detoxicator* for the excessive ammonia as suggested by Prianishnikov. The amide content in the $(NH_4)_2SO_4$ group are richer than those in the $NaNO_3$ group.

The similar results were obtained by Weissman(18) studying with wheat seedling, while he observed that the ration between the amides of asparagin and glutamin varied under the different conditions of nitrogenous culture; treated with ammonium there was more asparagin than glutamin; with nitrate, however, glutamin predominated. The present data, therefore, disagree with the work of Weissman because glutamin is always richer in the $(NH_4)_2SO_4$ than that in the $NaNO_3$ groups. The concentrations of 80% alcohol soluble nitrogen are well in agreement with the data of Walkley(22) working with barley seedlings treated with ammonium sulfate. Although Singh(11) and Syrett(12) found that there occurred a more rapid assimilation of NH_3 into soluble-N than that of NO_3 in Chlorella, on the other hand, Oland(6) observed the high content of soluble nitrogen in apple tree supplyed with NO_3. The present results also indicates the rapid assimilation of NO_3 into soluble-N as shown in Fig. 3. The speed of the formation of soluble nitrogen is accelerated somewhat by the urea foliar spray compared with the supply of urea through the root. Walkley(17) studying with barley seedling, showed that total nitrogen was swiftly increased by the supply of ammonium sulfate under nitrogen deficient condition. The present result agrees with his, though disagree with the data by Oland(6), who indicated the total nitrogen was more heightened by NO_3 applied through root than by urea sprayed on the leaves. As shown in Fig. 4, the author is interested in the fact that the content of total nitrogen in the urea series is the lowest on the 2nd day but the highest on the

4 th day.

This seems the reason why urea needs considerable period to be hydrolyzed and absorbed, and why the urea solution do not change the hydronium ion concentration with the absorption of the nutrients, so called "Physiological neutrality". Other cultivate solutions, when absorbed, change their hydronium ion concentration because they are not controlled artificially. At the end of the experiment the urea solution has pH 5.8, the $NaNO_3$ pH 6.6 and the $(NH_4)_2SO_4$ pH 3.8.

摘 要

葉面 撒布를 다룬 前報(4)의 結果와 比較하기 위하여 大麥根을 通하여 尿素를 주고 그 吸收와 代謝를 硏究하였고 아울러 尿素와 硝酸 及 硫安에 對해서도 比較하였다.

大麥幼植物의 硝酸의 最高含量은 尿素區는 가장 늦어 8日에, 硝曹區는 4日에, 硫安區는 尿素區와 비슷한 날에 나타났다. Ammonia의 最高含量은 尿素區는 他 兩區보다 2日 늦어졌고 葉面撒布해서 얻은 前報 보다는 4日 늦어졌다. Amide 含量은 窒素源에 따르는 差 或은 根에서 준 것과 前報의 葉面撒布의 結果가 모두 비슷한 傾向을 보여 주었다. 그러나 Glutamin은 Asparagin 보다 많은 含量을 보여 주었다. 80% alcohol 可溶窒素는 尿素區와 硝曹區는 같은 傾向이었고 硫安區의 最高含量은 前二區보다 빠르게 나타났다. 前報(4)와 比較하여 보면 根에서 尿素를 吸收시키면 葉에서 吸收시킨 것 보다 80% alcohol 可溶窒素의 含量도 亦是 늦어졌다.

總窒素含量은 尿素區는 初期에는 他區보다 낮은 含量을 가졌으나 4日後에는 오히려 높아졌다. 이것은 根을 通해서 尿素가 들어가기에는 硝酸이나 硫安보다 時間이 많이 걸리지만 尿素는 生理的 中性이기 때문에 pH가 變하지 않어서 吸收와 代謝의 障害를 받지 않는 것으로 생각된다.

LITERATURE CITED

Boynton, P., Margolis, D., and Gross, S.R.: Proc. Amer. Soc. Hor. Sci., 62:135, 1953.
Greenbill, A.W., and Chibnall, A.C.: Biochem. J., 28:1422, 1934.
Gregory, F.C., and Sen, P.K.: Ann. Botany, 1:(N.S.) 521, 1937.
Kim, J. H.: Korean J. Botany, 4:51, 1961.
Mothes, K.: Planta, 19:117, 1933.
Oland, K.: Physiol. Plantarum, 7:463, 1954.
_____: Physiol. Plantarum, 12:594, 1959.
Prianishnikov, D.: Ber., 40:242, 1922.
_____, and Schulow, P.: Ber., 28:253, 1920.
Schlenker, F.S.: Plant Physiol., 18:141, 1943.
Singh, R.N.: Nature, 165:325, 1950.
Syrett, P.J.: Ann. Botany (London), 17:1, 1953.
Tamm, C.O.: Physiol. Plantarum, 9:331, 1956.
Vickery, H.B., Pucher, G.W., and Clark, H.E.: J. Biol. Chem., 109:39, 1953.
_____, _____, Wakeman, A.J., and Leavenworth, C.S.: Conn. Agr. Esptl. Sta. Bull., : 399, 1937.
Viets, F.G., Whitehead, E. L., and Moxon, L. L.: Plant Physiol., 22:465, 1947.
Walkley, J.: New Phytologist, 39:362, 1940.
Weissman, G.S.: Amer. J. Botany, 46:339, 1959.
Yemm, E. W.: Proc. Roy. Soc. (London), 117:483, 1935
_____: Proc. Roy. Soc. (London), 123:243, 1937.