two cases, 30 days in one, 20 days in two and 14 days in another. Apparently life could have been maintained indefinitely with continued treatment. Dosages used varied from 0.5 to 5 mgm per day. The former was ineffective and the minimal life-maintaining dose seemed to be approximately 2 mgm per day. Marked weight gains occurred during treatment in 5 of 6 cases. The salt supplement usually added to the meat diet was not essential for the observed effects as determined by its omission in one case. All animals died of adrenal insufficiency when therapy was discontinued, showing that adrenalectomy was complete.

In two cases a commercial corpus luteum extract from hog ovaries proved toxic and reduced the life-span, perhaps because of estrin contamination.

Testosterone propionate, a substance chemically and in some ways biologically similar to progesterone, was slightly, if at all, effective in four animals, in doses of 5 or 10 mgm per day. The animals lived for 13, 12, 8 and 11 days. Similar results have been obtained in rats by others. Estrone given to two animals in doses of 0.1 and 0.2 mgm daily was toxic, as in the rat, and as was to be inferred from the effects of spontaneous estrus.

CONCLUSIONS

Crystalline progesterone will maintain adrenalectomized ferrets in excellent health and will relieve an established adrenal insufficiency. The phenomenon of life-maintenance in pseudopregnant adrenalectomized animals is therefore probably explained on the basis of progesterone secretion alone. Testosterone is non-toxic and non-beneficial to adrenalectomized ferrets, while estrone is toxic.

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NICOTINIC ACID AND THE GROWTH OF ISOLATED PEA ROOTS[1]

IN earlier publications[2,3] it has been shown that vitamin B_1 is an essential accessory factor for the growth of isolated pea roots. Pea roots were maintained for 14 weekly transfers in a medium containing only vitamin B_1 in addition to sucrose and mineral nutrients. It was also shown, however, that the growth rate of isolated pea roots decreases in successive transfers if vitamin B_1 is the only accessory growth factor. Such roots also become progressively thinner as the weekly transfers are continued. Vitamin B_1 alone is not then the only growth factor essential for the sustained growth of the isolated pea root. Other investigators have suggested that specific minor elements[4,5] or specific "essential" amino acids[6] constitute this second essential growth factor in the case of the isolated tomato root. The present authors also found that a mixture of 16 amino acids possessed activity as the second growth factor for the isolated pea roots.[7] The mixture of amino acids used in these earlier experiments was, however, a purely arbitrary one. In work which will be described in detail elsewhere, each individual amino acid was tested both alone and in combination with other amino acids (always in the presence of added vitamin B_1) for its ability to increase the growth of the isolated pea root above the level of growth rate of similar roots supplied with only vitamin B_1 as the accessory factor. In this way a mixture of amino acids having the composition shown in Table 1 and optimal for support of the growth rate of the isolated pea root was obtained. This mixture of amino acids was found capable of increasing the growth rate of isolated pea roots over the growth rate obtained with vitamin B_1 alone, but to be incapable of supporting growth at an undiminished rate for more than three weekly transfers. It is clear then that the second factor limiting the growth of isolated pea roots is not merely a combination of amino acids. It has also been found that the factor in question is not a specific minor element.

TABLE 1

AMINO ACID MIXTURE OPTIMAL FOR THE GROWTH OF PEA ROOTS

Amino acid	Concentration in medium in mg. per liter
Asparagine	0.1
Glycine	0.05
Glutamic Acid	0.5
Isoleucine	0.015
Leucine	0.5
Tryptophane	0.5
Valine	0.05

Nicotinic acid, in combination with vitamin B_1, exerts a very striking effect on the growth of the isolated pea root, as is demonstrated by the following experiment. Approximately 700 pea roots, which had been in culture on vitamin B_1 free medium for one week, were divided into 7 series. These separate series received as accessory growth factors either vitamin B_1, the amino acid mixture, nicotinic acid or combinations of these. The series receiving only vitamin B_1 will be considered the control. It may be seen in Table 2 that in all series in which nicotinic acid and vitamin B_1 were not both present there was a steady decrease of

[1] Report of work carried out with the assistance of the Works Progress Administration, Official Project No. 665-07-3-83, Work Project W-9809.

[2] J. Bonner, SCIENCE, 85: 183–184, 1937.

[3] J. Bonner and F. T. Addicott, *Bot. Gaz.*, 99: 144–170, 1937.

[4] W. J. Robbins and M. Bartley, SCIENCE, 85: 246–247, 1937; *Bot. Gaz.*, 99: 671–728, 1938.

[5] P. R. White, *Plant Physiol.*, 13: 391–398, 1938.

[6] *Ibid.*, 12: 793–802, 1937.

[7] J. Bonner and F. T. Addicott, *loc. cit.*

TABLE 2

EFFECT OF VITAMIN B_1, NICOTINIC ACID (0.5 MG PER LITER) AND "PEA ROOT AMINO ACID MIXTURES" DONE IN COMBINATION, ON THE GROWTH OF ISOLATED PEA ROOTS

Growth factors added	Growth in mms per week, average for 2 weeks		
	II and III	IV and V	VI and VII
B_1	41.1	23.6	11.6
Amino acids	32.5	22.1	5.9
Nicotinic acid	45.8	25.0	9.2
Nicotinic and amino acids	46.3	18.4	16.2
B_1 and amino acids	47.0	30.1	20.1
B_1 and nicotinic acid	55.8	70.6	72.6
B_1, nicotinic acid and amino acids	54.2	68.6	77.7

growth rate with each weekly transfer. Nicotinic acid alone, however, supported growth of the isolated pea root at least as well as did vitamin B_1. Combination of the amino acid mixture with either nicotinic acid or with vitamin B_1 supported the growth at a somewhat higher level than did either substance in the absence of the amino acid mixture. Combination of nicotinic acid with vitamin B_1 or with vitamin B_1 and the amino acid mixture, on the other hand, resulted in a steady increase of growth rate. This increase has been found in several different experiments and is of the order of 50 per cent. above the initial level. Although the experiment recorded in Table 2 was maintained through only 7 weekly transfers, a second experiment was maintained through 10 weeks with undiminished rate of growth of the roots which received nicotinic acid in addition to vitamin B_1 and the amino acid mixture (Fig. 1). Such roots were indistinguishable in appearance (except for their greater length) from roots which had been but 2 weeks in culture.

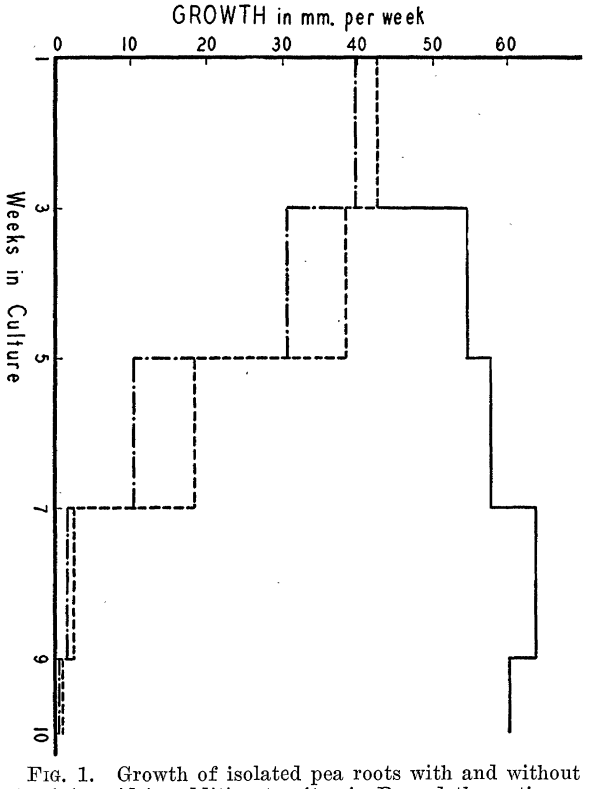

FIG. 1. Growth of isolated pea roots with and without nicotinic acid in addition to vitamin B_1 and the optimum mixture of amino acids: Solid line, nicotinic acid plus vitamin B_1 plus amino acids; dotted line, vitamin B_1 plus amino acids; dot and dash line, vitamin B_1 alone. Nicotinic acid was introduced into the medium of part of the series which had been cultured in vitamin B_1 and amino acids at the end of the third week. No accessory growth substances are added to the roots during the first week.

Although the data presented (in Table 2) do not yet permit of a definitive decision, still it seems possible that the amino acid mixture is not essential to the continued optimal growth of isolated pea roots. Nicotinic acid, however, must apparently be regarded as a factor quite as significant as vitamin B_1 in the nutrition of the isolated pea root. The promotive influence of nicotinic acid on the shoot growth of the isolated pea embryo[8] also must in all probability be attributed to the effect of this substance on the growth of the root.

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[8] J. Bonner, *Plant Physiol.*, in press.

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