We will refer to the mean level of activity in the different phenotypes as $\overline{A}$, $\overline{BA}$, $\overline{B}$, $\overline{CA}$ and $\overline{CB}$. If the effects of the three alleles P^a, P^b and P^c are additive then the following two relationships should occur:

$$(1)\ \tfrac{1}{2}\overline{A} + \tfrac{1}{2}\overline{B} = \overline{BA}$$

$$(2)\ \overline{CA} - \tfrac{1}{2}\overline{A} = \overline{CB} - \tfrac{1}{2}\overline{B}$$

From Table 2 it can be seen that: $\tfrac{1}{2}\overline{A} + \tfrac{1}{2}\overline{B} = 155{\cdot}35$; and that $\overline{BA} = 153{\cdot}9$; also $\overline{CA} - \tfrac{1}{2}\overline{A} = 122{\cdot}6$; and $\overline{CB} - \tfrac{1}{2}\overline{B} = 118{\cdot}15$. Thus the observed mean values give strong support to the hypothesis that the quantitative effects of these genes are additive in a simple way.

If this is so then from the results in Table 2 one may say that the average activity attributable to each of the three postulated genes will be approximately: P^a 61 units; P^b 94 units; P^c 120 units. One may note that the ratio of the activities $P^a : P^b : P^c$ is very close to 2 : 3 : 4, and it is tempting to think that this may have some special significance in terms of enzyme structure. Another point is that the values suggest that the predicted but not yet identified phenotype corresponding to the genotype P^cP^c should have an average activity of about 240 units. It will be interesting to see if this is confirmed when this rare phenotype is identified.

These findings are consistent with the patterns of activity in the different phenotypes as observed after starch-gel electrophoresis. Thus a pattern very similar to that observed in type BA has been obtained by running a mixture of approximately equal amounts of hæmolysates of types A and B. Also the patterns in types CA and CB can be plausibly regarded as equivalent to mixtures of a hypothetical type C with types A and B respectively. However, it should be noted that in types A and B, both of which are thought to represent homozygotes, two electrophoretically distinct zones of activity are present Furthermore, while in type A the two zones appear to be roughly equal in activity, in type B the faster zone is relatively more intense than the slower zone. A knowledge of the nature of the differences between the two enzyme components in these types, and also of the relative contribution each component makes to the total activity, would therefore be expected to provide further insight into the character of the gene dosage relationships involved.

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4-Aminobutyrate 1-^{14}C Metabolism in *Helianthus tuberosus* Tissues grown *in vitro*

4-Aminobutyrate (4-AB) has been found widespread in animals, plants and micro-organisms[1–4]. Because it is especially concentrated in brain[5], a more or less direct relation with neurotransmission mechanisms (as 4-aminobutyrylcholine[6], for example) has been suggested[7,8]. The transamination of 4-AB with 2-oxoglutarate to yield glutamate and succinic semialdehyde[9,10], resulting in a by-pass to the Krebs cycle, which has been shown in brain and in some micro-organisms, tentatively suggests a more general pathway[11]. Although 4-AB, as well as the enzyme that produces it from glutamate–L-glutamate 1-carboxylyase, are present in appreciable quantities in a high variety of plants[12], little is known about 4-AB metabolism in plant tissues.

Tissue culture in liquid medium appeared to be convenient for this purpose. Regular sized fragments of *H. tuberosus* tubers were placed in 200-ml. flasks each containing 10 ml. of a culture medium composed of glucose, mineral salts, auxin, thiamine and 345 mg/l. of 4-AB. The 4-AB 1-^{14}C was added in amounts ranging from 0·2 to 2 μc./ml. Agitation of 4 oscillations per min was applied (temperature, 23° C, illumination, 8 h a day). The flasks with 2 μc./ml. were able to retain the carbon dioxide evolved. Samples were taken each day for a week. Organic acid and amino-acid fractions were isolated by passing the hydroalcoholic extract of the tissues through ion-exchange resins ('Lewatit *S*–100'). Both fractions were concentrated *in vacuo* and the radioactivity of dry aliquots, plated on planchettes 19 mm in diameter, and of paper chromatograms spots measured (Tracerlab apparatus, 1,450 V; window of 2·5 and 1·8 mg/cm^2; background count rate, 12 c.p.m.).

The results obtained can be summarized as follows: (1) The radioactivity recovered in the organic acid and amino-acid fractions in the earlier samples shows the rapid incorporation of 4-AB in the tissues examined and an active metabolism of its carbon skeleton (Table 1).

Table 1. Incorporation of Labelled 4-Aminobutyrate in *H. tuberosus* Tissues grown *in vitro*

Days of culture	4-AB 1-^{14}C added (μc./ml.)	Recovered radioactivity (c.p.m.) in Amino-acids	Organic acids	$BaCO_3$ *
1	0·2	6·446	5·705	—
2	0·2	8·618	7·577	—
3	1	49·934	35·460	—
4	2	118·086	66·725	601·932
5	0·2	9·504	5·098	—
6	0·2	10·384	5·930	—
7	0·2	8·650	5·742	—

* Measured only in the 2 μc./ml. flasks.

(2) The amount of carbon dioxide, measured as barium carbonate, represents after 4 days' culture 78 per cent of the recovered radioactivity. In this preliminary work the radioactivity of insoluble material has not been taken into account. (3) In the chromatograms of the amino-acid fraction the highest values are found, in addition to 4-AB, in alanine, proline, aspartic acid and glutamic acid. (4) In the chromatograms of the organic acid fraction most of the radioactivity is found as malic acid. The remaining compounds have low or no activity.

These results are in agreement with those obtained in a simultaneous experiment carried out in our laboratory with glutamate 1-carboxylyase activity and amino-acid evolution in *H. tuberosus* grown in a medium where this amino-acid was the only source of nitrogen supply. A complete assimilation of 4-AB and a parallel increase between enzymatic activity and formation of new tissue have been found.

Therefore, a direct connexion between 4-AB and the Krebs cycle seems to be a reasonable explanation of the high rate transformations of 4-AB in *H. tuberosus* tissues, and suggests an active role in some steps of plant metabolism. It will be recalled that it is present in highly significant amounts in seeds, roots, tubers, etc.

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