STUDIES ON INTESTINAL ABSORPTION OF AMINO ACIDS

BY BROILER CHICKS AND RATS

by

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requirements of the University of Surrey for
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SUMMARY

The everted gut sac technique has been used to measure in vitro the absorption of amino acids by the intestine of broiler chicks, representing avian species, and of rats, representing mammalian species. Measurements of rates of absorption and concentrations gradients have been made in the chick at various ages from 5 to 56 days of age and in the rat from 21 to 60 days of age. Attempts have been made to correlate the findings with the amino acid requirements of the two species over these age ranges.

During the growth of the rat the rate of absorption of L-cysteine fell progressively to 60 days of age and that of methionine fell to 30 days whilst that of alanine changed little over the age range studied. At all ages the rate of absorption of cysteine was higher than that of methionine or alanine. The concentration gradient for cysteine was also always higher than that of either alanine or methionine.

The changes during growth in the rate of absorption of these three amino acids were similar in the chick to those in the rat. However, whilst the relationship between the concentration gradients of the different amino acids were similar, those in the chick changed little in their actual values during growth.

When rates of absorption of L-alanine were compared at various ages in rats and chicks it was found that the value in the rat fell gradually from 20 to 60 days but was at all ages higher than in the chick. In the latter species, the rate of absorption fell markedly between 5 and 11 days and by 30 days had fallen to a still lower value.
The rate of absorption of methionine was always higher in rats than in chicks and fell markedly between 5 and 11 days of age. In contrast, the concentration gradient for methionine rose sharply in the chick from 8 days of age to 19 days of age and from 11 days was always higher than in the rat. In the rat the gradient rose slightly between 21 and 30 days but changed little thereafter.

In contrast to the values for the absorption of methionine, those for cysteine were higher in the chick than in the rat. This was particularly true of the concentration gradient in which at maturity there was a three-fold difference. This species difference in particular may reflect the greater dietary requirement for cysteine in broiler chicks.

Oral use of antibiotics demonstrated no detectable difference in the rate of absorption and concentration gradient for methionine when these were offered for three consecutive days only. Results confirmed that a competitive relationship existed between glucose and methionine absorption for chicks. The rate of absorption for methionine was found to increase when glucose was absent from the perfusion medium. During growth rate studies the addition of further free sulphur amino acid to a diet containing only half the requirement of sulphur amino acid from natural ingredients demonstrated no effect on body weight and food utilization. The total amount of crude protein in this diet was 13.6% and metabolizable energy was 3007 Kcals/kg. In addition, when methionine or cysteine were offered separately, again no effect on body weight and food utilization was observed.

Basic studies indicated that active transport occurred throughout the whole hour of the incubation period, and that histological damage of the intestinal cells was insignificant.
ACKNOWLEDGEMENTS

I would like to thank Professor D V W Parke, Head of the Biochemistry Department, for providing facilities for this work. I am indebted to both Professor J W T Dickerson and Professor J B M Coppock OBE for their guidance and supervision with regard to the planning and execution and their helpful advice during the preparation of this thesis. I would like to thank Dr L T Jones for his helpful discussion concerning experiments included in this research.

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I am also very grateful to my wife for her continued moral support throughout the period of this work.

This study was supported by a grant from the Ministry of Higher Education, Republic of Iraq to whom I wish to express my sincere thanks.
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CHAPTER 1

INTRODUCTION
A. Poultry Production

(a) General considerations

The ability of the broiler to adapt to most countries of the world, the low economic value per unit, the rapid growth rate of the broiler, its composition, and finally the rapid generation time, makes it a valuable food. Poultry meat is a rich source of nutrients for human food. Broiler chicks can be produced for meat in seven weeks. It has been estimated that chicken appears in the diet of man as a source of meat more than any other form.

A brief history and characterization of the industry should help to point out the research developments and circumstances which brought about the poultry revolution.

Prior to 1930 most poultry was produced in small flocks as a side-line enterprise, usually to provide 'pin money' for the housewife. Only fresh poultry, of all types, was available. Since 1930, conditions and research developments in the fields of genetics, nutrition, disease control, management technology and marketing have made most countries in the world efficient as far as the poultry industry and meat production are concerned. The position is rapidly changing, and the importance of poultry production to other agricultural commodities is likely to increase further in the future. Several reasons can be suggested for its greater significance. One of these is the financial opportunity furnished by large-scale intensive production, a comparatively recent development in poultry farming. Another is the appreciation of the food value of poultry products, particularly with regard to protein, a dietary constituent often deficient in human nutrition in the developing parts of the world.
A further consideration which, it is expected, will increase the relative importance of poultry products, is the ability of large-scale production of broiler meat and eggs to proceed without the use of large areas of land. In places where a large population causes a pressure upon the available space, this can be an important consideration. However, it is a disadvantage of poultry that, in contradistinction to ruminants, they compete directly with human beings for much of their food supply. Also the process of conversion of these foods, such as cereals, into poultry, is to some extent wasteful so that one would hardly expect poultry to survive as starving conditions approach, except that it must be remembered poultry can be produced on scraps as evidenced currently in very poor countries.

Broilers are the main form of poultry meat production; Combs (1961) reported that more than 80% of the chicken consumed in the United States of America comes from broiler chicks. The Ministry of Agriculture, Fisheries and Food (1969) also reported that in 1953 the output of broilers in the United Kingdom was negligible. By 1960 production had reached an annual rate of 100 million birds and is currently running at about 360 million birds.

The very high rise in poultry production has started to be a major target in developing countries for the same reason as mentioned above. In Iraq, the home country of the author, there is a very high interest in the subject. A joint project, between six large European companies started in 1974 in Baghdad, showed the expected figures for broiler meat consumption per capita between the years 1975/1984 (Fig. 1.1). The figures show clearly the demand for broiler production. The aim of this rise is to reach the level of other countries in the world because of the nutritive value of broiler meat. The success of such a project
is dependent also on the feed development and production (Fig. 2.1), bearing in mind that poultry feed represents the highest percentage of total cost (in the range 60-70%) depending on the fluctuation of raw materials and the ingredients included in the diet.

It is worthwhile showing the latest report (1978) on broiler meat production and consumption per capita in different countries of the world (Table 1.1):

**TABLE 1.1 Poultry meat production and consumption in some countries**

<table>
<thead>
<tr>
<th>Country</th>
<th>Total poultry meat production (x 1000 tons)</th>
<th>Consumption kg/capita/year</th>
</tr>
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<tbody>
<tr>
<td>United States</td>
<td>5830</td>
<td>24.9</td>
</tr>
<tr>
<td>Canada</td>
<td>476</td>
<td>21.1</td>
</tr>
<tr>
<td>Spain</td>
<td>776</td>
<td>20.5</td>
</tr>
<tr>
<td>Italy</td>
<td>900</td>
<td>16.1</td>
</tr>
<tr>
<td>France</td>
<td>932</td>
<td>15.2</td>
</tr>
<tr>
<td>Czechoslovakia</td>
<td>230</td>
<td>14.6</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>754</td>
<td>13.6</td>
</tr>
<tr>
<td>Denmark</td>
<td>107</td>
<td>9.1</td>
</tr>
<tr>
<td>West Germany</td>
<td>330</td>
<td>9.1</td>
</tr>
<tr>
<td>Poland</td>
<td>340</td>
<td>9.0</td>
</tr>
<tr>
<td>Holland</td>
<td>355</td>
<td>7.0</td>
</tr>
<tr>
<td>Iran</td>
<td>-</td>
<td>6.5</td>
</tr>
<tr>
<td>USSR</td>
<td>1725</td>
<td>6.3</td>
</tr>
<tr>
<td>Brazil</td>
<td>728</td>
<td>5.6</td>
</tr>
<tr>
<td>Iraq</td>
<td>27</td>
<td>2.5</td>
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</table>
Poultry World (June, 1978) discussed the last 25 years of broiler production in the United Kingdom, and Fig.1.3 shows the growth of the UK broiler industry from virtually nothing in 1953 to around 360 million chickens in its twenty-fifth year. Table 1.2 shows the production of poultry feeds in the European Economic Community from 1972 to 1977.

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<td>United Kingdom</td>
<td>3.87</td>
<td>3.82</td>
<td>3.50</td>
<td>3.36</td>
<td>3.46</td>
<td>3.35</td>
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<tr>
<td>West Germany</td>
<td>3.65</td>
<td>3.58</td>
<td>3.50</td>
<td>3.48</td>
<td>3.34</td>
<td>3.28</td>
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<td>2.16</td>
<td>2.23</td>
<td>2.25</td>
<td>2.18</td>
<td>2.30</td>
<td>2.35</td>
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<tr>
<td>France</td>
<td>3.27</td>
<td>3.75</td>
<td>3.88</td>
<td>3.81</td>
<td>4.00</td>
<td>4.06</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>1.12</td>
<td>1.11</td>
<td>1.17</td>
<td>1.02</td>
<td>0.98</td>
<td>1.08</td>
</tr>
<tr>
<td>Italy</td>
<td>1.50</td>
<td>2.88</td>
<td>2.58</td>
<td>2.53</td>
<td>2.63</td>
<td>3.55</td>
</tr>
<tr>
<td>Denmark</td>
<td>0.60</td>
<td>0.61</td>
<td>0.59</td>
<td>0.55</td>
<td>0.56</td>
<td>0.55</td>
</tr>
<tr>
<td>Republic of Ireland</td>
<td>0.27</td>
<td>0.27</td>
<td>0.25</td>
<td>0.24</td>
<td>0.26</td>
<td>0.21</td>
</tr>
</tbody>
</table>

B. PROTEIN, ENERGY AND AMINO ACIDS

(a) Protein energy ratio

It is very difficult to set an energy requirement in terms of kilocalories per kilogram of diet because chicks adjust their feed intake to achieve the necessary daily ration of energy. Protein requirement, on the other hand, is one of the very important criteria
FIG. 1.3 Annual output of chicken showing the growth of the UK broiler industry from 1953-1978 (after Poultry World, June 1978)
upon which any feed formula is based. If it is to be specified, the dietary energy level must also be specified for it is essential to maintain the proper ratio of protein to energy in poultry diets. Some variability in optimal protein:energy ratios must be recognised. Some combinations of fats and carbohydrates have a protein-sparing effect. The ratio of protein to energy may be altered on purpose in order to influence fat deposition. When protein levels are low in relation to energy with ad libitum feeding, fat deposition is markedly increased; when higher protein levels are used, less fat is deposited. Increasing the level of protein above that required for maximum growth rate reduces fat deposition further.

It is evident that the protein requirements can be defined accurately only in relation to energy concentration, the degree of fat deposition, and a limited range of nutrient combinations, using those practical foodstuffs which have been subjected to experimental study. For practical reasons in farm operations and feed manufacturing, sufficient work has been done with growing chicks to define, with reasonable accuracy, the minimum protein requirement for maximum growth rate in relation to energy level. The physiological relationship between levels of energy and protein extends also to the level of the essential amino acids. Combs (1955) brought attention to information concerning the relationship of protein to energy and its importance in broiler nutrition. Some of his results are shown in Table 1.3. These figures are presented from several experiments and show the effect of the calorie-protein ratio on the body weight of the broiler chicks at four weeks. Scott et al (1947) and Singsen and Matterson (1950) also demonstrated that high 'energy' diets permitted broilers to grow more rapidly and use their feed more efficiently.
TABLE 1.3 Modified table for metabolisable energy-protein ratio studies: broiler chicks (after Combs, 1955)

<table>
<thead>
<tr>
<th>Protein %</th>
<th>ME*** kcal/kg</th>
<th>ME** M J/kg*</th>
<th>P/MJ Ratio**</th>
<th>4-week wt. (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.9</td>
<td>2120</td>
<td>8.87</td>
<td>2.92</td>
<td>471</td>
</tr>
<tr>
<td>23.2</td>
<td>2136</td>
<td>8.94</td>
<td>2.60</td>
<td>475</td>
</tr>
<tr>
<td>25.8</td>
<td>2416</td>
<td>10.11</td>
<td>2.55</td>
<td>490</td>
</tr>
<tr>
<td>25.8</td>
<td>2658</td>
<td>11.12</td>
<td>2.32</td>
<td>471</td>
</tr>
<tr>
<td>20.0</td>
<td>2131</td>
<td>8.92</td>
<td>2.24</td>
<td>427</td>
</tr>
<tr>
<td>20.6</td>
<td>2394</td>
<td>10.02</td>
<td>2.06</td>
<td>452</td>
</tr>
<tr>
<td>20.6</td>
<td>2645</td>
<td>11.07</td>
<td>1.86</td>
<td>425</td>
</tr>
<tr>
<td>17.9</td>
<td>2660</td>
<td>11.13</td>
<td>1.61</td>
<td>389</td>
</tr>
</tbody>
</table>

* 4.184 J = 1 cal
0.004184 MJ = 1 kcal

** P/MJ = \frac{\text{Crude protein}}{\text{ME (MJ/kg)}}

*** Metabolisable energy

Soon after, Schweigert et al (1952) were successful in using an antioxidant to stabilise 'inedible' fat, thus permitting its use in rations for poultry. Excellent chick growth has been obtained with rations containing as little as 505 kcal (Hill and Dansky, 1954), and as many as 1959 kcal (Combs et al, 1958) of productive energy per pound. The satisfactory use of rations containing such markedly different levels of 'energy' illustrates the need for expressing the nutrient requirement in terms other than as a function of the weight of the diet.

It is generally considered that for any particular type and intensity of physiological function a specific amount of each amino
acid is required. It follows that the requirement expressed as a percentage of the diet will vary with the amount of diet eaten, which in turn will be related to the concentration of energy in it. In most experiments the amino acid pattern has been kept constant apart from the amino acid under test, and amino acid and energy levels of the diets have been estimated from tables, whilst optimal values have not been determined experimentally. The energy level has been altered intentionally in only a few studies with lysine and methionine (Baldini et al, 1955, 1957; Schwartz et al, 1958) for which the relationship between the requirement and the energy level of the diet has been experimentally established (Table 1.4), but such data is not available for the other essential amino acids.

TABLE 1.4 Lysine and sulphur amino acid requirements of chicks (0-4 weeks) at different energy levels in the diet (after Schwartz et al, 1958; Baldini and Rosenberg, 1955)

<table>
<thead>
<tr>
<th>Amino acid (g/100 g)</th>
<th>Metabolizable energy level (M cal/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.4</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.9</td>
</tr>
<tr>
<td>Methionine + Cystine*</td>
<td>0.6</td>
</tr>
</tbody>
</table>

* The evidence available indicates that at least 40% should be in the form of methionine.

Poultry World (November, 1978) presents an up to date reference, Table 1.5, showing the nutritional constraints for the broilers' feed. Energy values are given in both kilocalories per kilogram and in
TABLE 1.5 Nutrient requirement for broiler chicks (after Poultry World, November 1978)

<table>
<thead>
<tr>
<th></th>
<th>Crude Protein</th>
<th>ME Kcal/kg</th>
<th>ME MJ/kg</th>
<th>Lysine</th>
<th>Methionine</th>
<th>Ca.</th>
<th>Available phos.</th>
<th>Salt max.</th>
<th>Na (Min.)</th>
<th>Essential fatty acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Super starter*</td>
<td>23</td>
<td>3040</td>
<td>12.7</td>
<td>1.30</td>
<td>0.60</td>
<td>1.0</td>
<td>0.45</td>
<td>1.40</td>
<td>0.12</td>
<td>0.80</td>
</tr>
<tr>
<td>Starter**</td>
<td>21</td>
<td>3040</td>
<td>12.7</td>
<td>1.15</td>
<td>0.57</td>
<td>1.0</td>
<td>0.45</td>
<td>0.40</td>
<td>0.12</td>
<td>0.80</td>
</tr>
<tr>
<td>Grower***</td>
<td>19</td>
<td>3080</td>
<td>12.9</td>
<td>1.00</td>
<td>0.50</td>
<td>1.0</td>
<td>0.43</td>
<td>0.40</td>
<td>0.10</td>
<td>0.60</td>
</tr>
<tr>
<td>Finish</td>
<td>18</td>
<td>3080</td>
<td>12.9</td>
<td>0.90</td>
<td>0.45</td>
<td>1.0</td>
<td>0.40</td>
<td>0.40</td>
<td>0.10</td>
<td>0.50</td>
</tr>
</tbody>
</table>

* 500 g/bird
** 1000 g/bird
*** 1500 g/bird
megajoules per kilogram. These rations show the relative balance of the nutrients to each other. Where an energy level different to that in the table is used, all the other nutrients should be adjusted accordingly.

Since protein-energy relationships show that there is a practical method improving broiler diets, considerable research has been carried out in this field. It was of interest to find out whether or not different types of animal fats could give this energy response. Many workers have shown that the quality of the fat itself, as long as there is sufficient antioxidant present in the diet, is unimportant. In other words, high-quality fat is no better from an energy standpoint than the poorest quality animal fat available from commercial sources.*

It has been shown by many workers that hydrogenation of fat does not slow the rate of absorption. The sole use of hydrogenated fats is not advocated in broiler diets.

Food conversion ratio appears to be very important in poultry broilers in particular. Increasing food utilisation will result in a higher conversion ratio. Scott _et al_ (1969) recommended the methionine requirement for best growth and maximum food conversion of a common strain of broiler chicks. Also, Sekiz _et al_ (1975) showed interesting aspects of the studies with methionine and observed that a higher level of dietary methionine is required to produce maximum rate of growth. When providing the broiler chicks with 18% protein in a commercial diet, 45% soybean meal was the source of protein. Methionine content in the mentioned diet was 0.25%. Sekiz _et al_ also state, "The chicks overeat when fed a diet slightly deficient in

* Some experts also considered acidity to be detrimental to performance. This is now known to be largely untrue.
methionine, thereby attaining near-maximum growth but bringing about a decrease in feed utilization".

Breed and feed are the most effective factors in the broiler industry. It is important to select the type and strain of chickens to obtain maximum growth and the best edible meat. Quite a number of workers have reported work dealing with comparisons of meat yields between breeds, strains and crosses of broiler. Male broiler chickens always tend to grow heavier than females; this indicated the sexual dimorphism, and explains the reason why all broiler producers supply male chickens for meat production.

(b) Energy balance

The fate of the gross energy of the food has been explained by King and Farner (1961) as follows:

\[
\text{Gross energy} \rightarrow \text{Digestible energy} \rightarrow \text{Net energy}
\]

\[
\downarrow \quad \downarrow
\]

\[
\text{Faecal waste} \quad \text{Urinary waste}
\]

\[
\text{Metabolizable energy} \rightarrow \text{Net energy}
\]

\[
\downarrow
\]

\[
\text{Specific dynamic action}
\]

Part of the energy contained in the substances absorbed from the gut (the digestible energy) is excreted largely by the kidney as uric acid. The energy retained in the body is the metabolizable energy, amounting to between 70% and 90% of the gross energy, depending on diet, environmental temperature, species of bird, and other factors. Not all of the metabolizable energy is available for growth,
maintenance, performance of work or storage, or for other special functions such as egg production. The absorption of energy from the gastrointestinal tract is followed, soon after its absorption, by an increase in heat production. The heat is referred to variously as the heat increment, the calorigenic effect, or the specific dynamic action (SDA) of the diet. The heat is thought to be derived from the exothermic reactions associated with the metabolism of the absorbed food molecules.

The net energy represents the metabolizable energy less the specific dynamic action. If the bird performs work, some of the net energy is transformed into work; but the inefficiency of this transformation is such that some of the energy appears as heat. If no work is performed and if the body weight, composition and temperature do not change, then all the metabolizable energy appears as heat.

(c) **Protein and amino acid requirements**

Protein requirements actually represent requirements for the essential amino acids and for sufficient nitrogen for biosynthesis of the more essential amino acids needed for optimum growth and production. Essential amino acids are those which cannot be synthesized by the individual, or only at too low a rate, so that they have to be supplied in the diet. Those amino acids which can be synthesized at a sufficient rate, and thus do not limit the metabolic processes, have been classified as dietary non-essential amino acids (see Table 1.6):
<table>
<thead>
<tr>
<th>Essential amino acids</th>
<th>Non-essential amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>Methionine</td>
</tr>
<tr>
<td>Cystine*</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Histidine</td>
<td>Threonine</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>Leucine</td>
<td>Tyrosine**</td>
</tr>
<tr>
<td>Lysine</td>
<td>Valine</td>
</tr>
<tr>
<td></td>
<td>Alanine</td>
</tr>
<tr>
<td></td>
<td>Aspartic acid</td>
</tr>
<tr>
<td></td>
<td>Glutamic acid</td>
</tr>
<tr>
<td></td>
<td>Glycine***</td>
</tr>
<tr>
<td></td>
<td>Proline</td>
</tr>
<tr>
<td></td>
<td>Serine</td>
</tr>
</tbody>
</table>

* Cystine can replace 50% of the methionine (DSM, 1966).

** Tyrosine can replace the phenylalanine requirement, as long as the ration contains not less than 0.9% phenylalanine (Morrison, 1959).

*** The chick can synthesize glycine, but not at a rate sufficient for maximum growth. Glycine can be replaced by serine and partially by arginine (Morrison, 1959).

The non-essential amino acids are nutritionally only important in that they provide the chicks with nitrogen further needed for protein and tissue synthesis. Generally, the proteins provide sufficient amounts of these non-essential amino acids. However, the classification of the amino acids into essential and non-essential categories apparently only has significance if related to the age and reproductive stage of the chicks, because these factors change the ability to synthesize amino acids and also have an effect on the assortment and the amount of essential amino acids that must be present in the diet. The poultry requirements for essential amino acids also depend to a large extent upon the energy content of the diet. Because a chicken consumes only that amount of feed each day
needed to meet its energy requirement, it is necessary that the amino acid values in the feed be such that the animal receives in its daily food intake an exact amount of each of the essential amino acids, plus sufficient non-specific nitrogen for optimum synthesis and deposition of tissue. Deficiencies, excess or imbalance of essential amino acids results in wasteful decreases in the efficiency of food utilization.

The concept of the amino acid balance was formulated by Harper (1958, 1959), on the basis of the relationship between the amino acid pattern of a protein and its nutritive value. Provided that a protein in which the essential amino acid pattern reasonably meets the amino acid requirements of the bird, it has been proved that optimum performance can be obtained when fed at somewhat lower levels of total protein. Perhaps the best method of expressing amino acid requirements is in terms of the energy content of the diet. However, since most of the essential amino acid requirements are supplied in the form of feed proteins, it is usually most useful to express the amino acid requirements as definite percentages of the dietary protein, and then to adjust the level of this protein according to the energy content of the diet.

(d) More detailed consideration of amino acid requirements

The amino acid requirements of broiler chicks have been determined in contrived experimental conditions in which the requirements for a single amino acid is determined. A basal diet is chosen which is intended to be adequate and supply the requirement of all amino acids, except the one under study. Different levels of this single amino
Acid are added to the basal diet to determine the minimum amount required for the maximum or minimum level of the response. Growth rate and food consumption per unit weight gain are used as the criteria. Other workers (e.g. Klain, Scott and Johnson (1960); Dobson, Anderson and Warnick (1964); and Dean and Scott (1965), have assessed the requirements of chicks for essential amino acids in relation to the level of protein in the diet. Again the effects of different levels of amino acids on weight gain and food utilization were examined.

There are many factors affecting the amino acid requirements for growing chicks. Table 1.7 summarises the work on amino acid requirements and is represented by the values of Dean and Scott (1965), Agricultural Research Council (1963), National Research Council (1966, 1971), Hewitt and Lewis (1972), and Scott (1972). The current values for the later work done by Hewitt and Lewis (1972) are probably due to the achievement of a good balance between amino acid levels which show lower requirements for most amino acids.

A large number of reports have been published on the subject of avian dietary requirements of amino acids and in particular the methionine and total sulphur amino acid requirement and their supplementation. Some of the factors contributing to the difficulties of ascertaining these requirements are that most workers use the calculated and not assayed values of methionine content in the feed stuffs. There is also a great variability of the assumed values employed in these calculations (Wilgus, 1958). In addition there is the problem of biological availability of sulphur amino acids in different dietary constituents (Evans et al., 1956), and the dependence of the methionine requirement on the amount and kind of dietary
TABLE 1.7 Reported values for the amino acid requirements of growing chicks as a percentage of the diet

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>0.55</td>
<td>0.65</td>
<td>0.70</td>
<td>0.80</td>
<td>0.74</td>
<td>0.53</td>
<td>0.75</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.00</td>
<td>1.60</td>
<td>1.00</td>
<td>1.15</td>
<td>0.60</td>
<td>0.61</td>
<td>1.50</td>
</tr>
<tr>
<td>Valine</td>
<td>0.80</td>
<td>0.82</td>
<td>0.85</td>
<td>1.00</td>
<td>0.90</td>
<td>0.79</td>
<td>0.82</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.50</td>
<td>0.80</td>
<td>0.75</td>
<td>0.86</td>
<td>0.84</td>
<td>0.61</td>
<td>0.80</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.50</td>
<td>1.20</td>
<td>1.40</td>
<td>1.60</td>
<td>1.47</td>
<td>1.34</td>
<td>1.35</td>
</tr>
<tr>
<td>Phenylalanine (Phe)</td>
<td>0.60</td>
<td>0.68</td>
<td>0.70</td>
<td>0.80</td>
<td>0.74</td>
<td>0.72</td>
<td>0.72</td>
</tr>
<tr>
<td>Phe + Tyrosine</td>
<td>1.20</td>
<td>1.31</td>
<td>1.30</td>
<td>1.50</td>
<td>1.27</td>
<td>1.34</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>1.00</td>
<td>1.12</td>
<td>1.10</td>
<td>1.25</td>
<td>1.05</td>
<td>0.85</td>
<td>1.20</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.35</td>
<td>0.30</td>
<td>0.40</td>
<td>0.46</td>
<td>0.42</td>
<td>0.40</td>
<td>0.35</td>
</tr>
<tr>
<td>Arginine</td>
<td>-</td>
<td>1.10</td>
<td>1.20</td>
<td>1.40</td>
<td>1.26</td>
<td>0.85</td>
<td>1.44</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.15</td>
<td>0.225</td>
<td>0.20</td>
<td>0.23</td>
<td>0.21</td>
<td>0.17</td>
<td>0.23</td>
</tr>
<tr>
<td>Methionine (Meth)</td>
<td>0.28</td>
<td>0.45</td>
<td>0.40</td>
<td>0.46</td>
<td>0.42</td>
<td>0.39</td>
<td>0.50</td>
</tr>
<tr>
<td>Meth + Cystine</td>
<td>0.70</td>
<td>0.80</td>
<td>0.75</td>
<td>0.86</td>
<td>0.75</td>
<td>0.79</td>
<td>0.94</td>
</tr>
</tbody>
</table>

* Providing the total protein in the final diet is 20.8% and the metabolizable energy 2755 kcal/kg.

** Requirement for diets containing 23% protein in the final diet and 3200 kcal metabolizable energy per kg.
protein (Gran and Kumei, 1950), and variability in the requirement of methionine (Hess et al, 1962).

The effect of source and manufacturing methods on the sulphur amino acid content of processed animal and plant proteins, mostly oxidation of methionine to methionine sulfoxide also contributes. There is an effect of environmental temperature of basal diet on the methionine requirement (Camp and Couch, 1959). Efficiency of absorption of D-, L and DL-methionine from the gut (Katz and Baker, 1975) is another factor.

Finally, the interrelationship between methionine and cystine as far as requirement is concerned: the National Research Council (1977) stated that the requirement of methionine can be met only by methionine, while the requirement for cystine may be met by cystine or methionine. This is because methionine is readily converted to cystine metabolically, while the reverse is not possible.

The physiological relationship between levels of energy and protein extends also to the levels of the essential amino acids. Ideally, the diet should provide energy, total protein and all essential amino acids in the amounts required depending on the age and type of bird. Because of the limited number of protein supplements, each of which has its own fixed ration of essential amino acids, this is too much to expect. The major sources of energy and protein for most poultry feeds are grains such as barley and wheat, together with soya seeds, cotton seed and peanuts. In such feeds, methionine is the first amino acid to become limiting. In agreement with Miller and Donoso (1963) Swendseid and Wang (1970) demonstrated that 'methionine (in the essential sulfur amino acids) is the limiting amino acid of
many common feeds and food", other than the cereal grains. The methionine requirement of broiler chicks is affected by many factors. Nelson et al. (1960) found that methionine needs of young chicks can be affected by the protein and energy level of the diet. Also Carew and Hill (1961) studied the effects on energy utilization of food intake and moderate deficiency of sulphur amino acids in the diet of chicks. Harms and Damron (1969), Fisher and Morris (1970), Roberson et al. (1970) and Hewitt and Lewis (1972b) also have studied the requirement of the chick for sulphur amino acids.

It is generally accepted that the levels of amino acids in diets fed to chicks affect their level in the blood. Further, as the blood vascular system is responsible for the transport of amino acids around the body, it is to be expected that the levels of amino acid in blood will be affected by the utilization of amino acids for protein synthesis. The actual level of each amino acid in blood will, to a large extent, be the result of a balance between these two factors. Therefore, consideration must be given to the use of blood plasma amino acid level determination in assessing the quality of the balance between the levels of individual amino acids in the diet. The important reason for this approach is the development of chemical methods for estimating nutritionally available methionine. A chemical procedure might offer advantage of speed and economy over the existing enzymic, microbiological and animal methods (Shorrock and Ford, 1973; Scott and Smith, 1966; Ford and Salter, 1966; Miller et al., 1965a; Carpenter et al., 1972). Such quick chemical methods ultimately would have to be correlated with biological estimates of nutritional availability in the species for which the nutrient was intended. Important factors must be considered carefully while processing
protein sources, such as the fact that heat damage to proteins reduces the availability of methionine (Clandinin et al, 1947; Evans and Butts, 1949; Miller et al, 1965b), but there has been no precise definition in terms of the responsible chemical changes. The general supposition (Ford, 1973) is that loss of availability of amino acids is due to interactions which involve amino acid functional groups. Such interactions may occur between functional groups of protein chains alone or between these functional groups and other food constituents, particularly carbohydrates, or with chemical additives during the heat treatment of the diet as in commercial practice. The resultant new linkages are presumed to decrease the utilization of the affected amino acid.

Food conversion ratio is very important in poultry broilers. Increasing food utilization will result in a higher conversion ratio. Scott et al (1969) recommended the methionine allowance for best growth and maximum food conversion of a common strain of broiler chicks. Also, Sekiz et al (1975) showed interesting aspects of the studies with methionine and observed that a higher level of dietary methionine is required to produce maximum food growth.

The biological availability of methionine can be reduced due to its conversion to methionine sulphoxide. This has been studied by a number of investigators with varying results using a variety of methods ranging from simple growth assay (Bennet, 1939), to measurement of net protein utilization (Miller, 1968). All these experiments depend on the use of proteins containing marginal amounts of methionine to which was added a supplement of methionine or its sulphoxide. Miller et al (1970) in experiments on rats 1 day old to 17 days of age showed that there were
significant differences in body weights and food efficiencies between
the group taking methionine sulfoxide and the group taking methionine
(both mixed with their basal diet and each at the same level). They
also showed that, in the long-term, rats tend to adapt to methionine
sulfoxide with age, permitting increased utilization of this compound
or a decreased need for methionine as the animals equally increase in
age and size.

However, food efficiencies could be very good as an indicator to
study the ability of the animal to take methionine sulfoxide. On
the other hand, the findings of Miller et al (1970) do not confirm the
suggestion of Njaa (1962), and Ellinger and Palmer (1969) that the
decreased availability of methionine in fish meal is the result of
oxidation of the sulfoxide making the peptide linkage involving
methionine less available for proteolysis.

Food and its dietary components are of no direct value until they
are absorbed, and some of the factors influencing these processes will
now be considered.

C. **INTESTINAL ABSORPTION**

(a) **Physiology of intestinal absorption**

In an investigation of the absorption of a substance from the
intestinal lumen one would like to be able to obtain results under
several headings, e.g. (1) disappearance of the substance from the
lumen, (2) appearance of the substance in the blood, (3) accumulation
of the substance in the intestinal wall, (4) metabolism of the
substance by the intestine, (5) gaseous metabolism of the intestine during the absorption, (6) effect of other added substances, and (7) activity of different regions of the small intestine.

Many studies have been carried out on animals to determine the influence of various types of dietary stress on the absorptive capacity of the intestine. Friedman (1953) suggested that, in the small intestine, the tissue layer which is affected by variations in the diet is the mucosal epithelium, or perhaps the whole of the mucosa. The overall structure of the alimentary canal for chick and rat are shown in Chapter 2 (Figs. 2.1 and 2.2).

The small intestine is mainly responsible for the absorption of nutrients and largely responsible for the absorption of water and sodium. In the upper small intestine, transit is rapid but the rate slows progressively as the lower ileum is approached. The ability to absorb many foodstuffs including protein, fat and carbohydrate, is good or particularly good in the upper part. For this reason, many substances are largely absorbed in the upper small gut (Booth, 1967). Important exceptions are vitamin B\textsubscript{12} and the reabsorption of bile salts which are absorbed in the lower ileum. Fat-soluble substances are absorbed mainly into the lymphatic system.

The response to both the quality and the quantity of nutrients ingested (Figs. 1.4 and 1.5) affords the first opportunity for metabolic adaptability, thereby conditioning the metabolic environment of the tissues concerned.

(b) Structure of the absorptive cells

The small intestine comprises four main substructures (Fig. 1.6):
FIG. 1.5 Intestinal absorption of long and medium-chain triglycerides

Tg : triglyceride
LCT : long-chain triglyceride
MCT : medium triglyceride
FA : fatty acid
FIG. 1.6  Structure of the small intestine of the mammalian.  
1. The innermost mucosa (facing the lumen of the intestine).
2. The submucosa coat.
3. The circular muscle.
4. The longitudinal coat.

The cells of the mucosal epithelial layer, through which absorption takes place, line the villi and consist of the primary absorptive columnar cells and a number of mucus-secreting cells, both of which are constantly renewed in the crypts. This turnover of absorptive cells contributes part of the endogenous protein in the lumen of the tract.

The absorptive columnar cells rest on a basement membrane which separates them from the lumina propria (connective tissue between the epithelium and the muscularis mucosae). The lamina propria constitutes the core of the villus surrounding their blood capillaries and lymphatic vessels. It is suggested that the basement membrane does not act as a permeability barrier (Matthews, 1968), and may contain fenestrations.

The microvilli on the brush border of the luminal face of the absorptive columnar cells are about 1 μ long by 0.7 μ wide, and there are between 1000 and 3000 microvilli per cell. The brush border of the cell is separated from the body of the cell by the terminal web. The terminal web is a meshwork of fine filaments and may serve to strengthen the region at the base of the microvilli. At the level of the terminal web each cell is joined to the adjacent ones by the fusion of the cell membrane in a structure, the tight junction, which connects the cells into a continuous sheet. Laterally, and near the basement membrane, the membranes of the adjacent cells separate to
form a lateral intercellular space.

(c) **Membrane structure of the absorptive cells**

The brush border contains high concentrations of hydrolytic enzymes which include disaccharidases, aminopeptidases and alkaline phosphatase associated with the plasma membrane of the microvilli. This membrane consists of lipid molecules between two layers of protein molecules (Pyke and Brown, 1967). Attached to the outer surface of the membrane is a coating of matted, radially arranged, fibrils which consist of sulphated mucopolysaccharide and is known colloquially as the "fuzz coat". The role of the fuzz coat is doubtful, but it may help to exclude unwanted particles such as bacteria, or to bind substrates which are to be absorbed. The lipid layer of the membrane is penetrated by minute water-filled pores occupying 0.1% or less of the surface. These aqueous pores have not been seen with the electron microscope but there is evidence suggesting their existence (Matthews, 1968). The mucosal membrane is endowed with distinctive morphological specialisations which facilitate absorption (Trier, 1967).

Superficial columnar epithelial cells, one cell thick, compose the mucosal lining, these being interspersed with variable numbers of mucin-secreting goblet cells, and a much smaller number of Paneth and Argentaffini cells occurring in the crypts between the villi. These villi, or tongue-shaped extrusions of the lamina propria, present to the intraluminal contents, a very much greater absorptive surface layer, further multiplied by folds in the duodenum and jejunum and by myriad microvilli comprising the brush border of each cell.
The multiple transport processes carried out by the mucosal epithelial cells of the small intestine have been worked out and appreciated by many investigators (for example, Wilson, 1962; Crane, 1960, 1965, 1968; Wiseman, 1968).

Most of these studies have been performed in such a way as to provide rather indirect information about the transfer form in which metabolites move from a compartment of low concentration to one of higher concentration, i.e. movement across the plasma membrane barrier of the epithelial cell. Furthermore, it is becoming increasingly clear that transport across intestinal mucosal cells may be similar to that occurring in cells of other tissues, and many workers focus on this possibility (Vidaver, 1964; Kleizeller et al., 1967a,b; Eddy, 1968; Koser and Christensen, 1968; Schultz and Curran, 1969).

Few studies have been made on intestinal absorption in the avian but it is presumed that the rate of absorption is fast because of the higher metabolic rate, body temperature and circulation time in comparison to most mammals. Emslie and Henry (1933) demonstrated the absorption of glucose, galactose and lactose in young chicks. These investigations found the three sugars to be absorbed at the following relative rates: glucose > galactose > lactose. Golden and Long (1942) reported that glucose absorption in the chicken was indeed rapid. Aramaki and Weiss (1962), who determined the concentration of glucose and amino nitrogen in portal venous blood and wing vein blood, showed that within 15 minutes after eating there was a significantly higher concentration of these substances in portal blood, indicating a high rate of digestion and absorption. Also, Conrad and Scott (1942) showed that the rate of absorption of fatty acids via the portal vein
ranged from 1.1 to 2.0 millimoles per hour. Williams and Fuller (1971) showed that high levels of ammonia in the diet depress chick growth. One possible reason for this is suggested by the work of Chow et al. (1972) who proved that ammonia, in the small intestine of the chick, inhibited the absorption of glucose, calcium and phosphorus.

D. MECHANISMS OF ABSORPTION

(a) Transport mechanisms of substances

During absorption through the intestine a substance must cross the plasma membrane of the absorptive cell, and much attention has been given to the problems of membrane transport (Curran et al., 1967). The substance could cross the membrane of the cell in several ways, namely:

(i) If the substance is lipid-soluble it crosses by diffusion through the membrane itself.

(ii) If the substance is water-soluble and its molecules are small enough, it passes through the aqueous pores.

(iii) By a carrier mechanism: such mechanisms probably involve a protein carrier in the cell membrane, and appear to be responsible for the transport of many water-soluble substances which are too large to go through the aqueous pores such as glucose, amino acids and also certain ions (Matthews, 1968). The carriers, which are specific for different substrates, may combine with the substrate molecule at one side of the membrane and release it at the other side.
Anatomically, the intestinal tissue reflects a specific role which is further emphasised by its biochemical apparatus. An excellent summary which deals with the knowledge of the gut mucosal enzymes has been provided by Spencer and Knox (1960). The distribution of the enzyme pattern appears to change along the length of the intestine, and also to alter in relation to the changing state of the whole animal. The process is called 'adaptation'. Such a dynamic relationship between diet, function and form of the gut mucous membrane cannot be over-emphasised. It is obvious, but still largely unrealised, that correlation of induced enzymic changes in the gut, with altered physiological state of the animal, and changes in the absorptive capacity of the intestine, occur at the same time. This offers an important approach in identifying the basis of the functional activity of the intestine.

Three main types of transport are recognised for transporting the substrate, namely:

1. Active transport.
3. Facilitated diffusion.

1. Active transport

Active transport depends on metabolic energy in order to move the substrate against an electrochemical gradient (Hagihira et al, 1961; Wiseman, 1955). A carrier mechanism appears to be involved in this metabolic process. The existence of active transport is demonstrated by showing that the substrate can be moved against electrochemical and concentration gradients. Active transport plays a role in the absorption of many nutrients including sodium, amino acids, some
monosaccharides, calcium, iron and some vitamins. Solid evidence is provided by showing that the transport is abolished by cutting off the metabolic energy supply, by such means as anoxia, metabolic inhibitors, or reducing the temperature.

L-amino acids were reported (Elsden et al, 1950) to be selectively absorbed by active transport. The concentration of any free individual amino acid within the lumen after a protein meal will be affected by several factors. These include (1) the rate of release of free amino acids by digestive enzymes, for example lysine and arginine are rapidly released by the combined actions of trypsin and carboxypeptidase B; tyrosine and phenylalanine by pepsin, chymotrypsin and carboxypeptidase-A (Dixon and Webb, 1964); (ii) the extent to which amino acids liberated by intracellular peptidases diffuse back into the lumen. Evidence that this can be variable has already been quoted; (iii) the rate of absorption of the free amino acid.

(2) Passive diffusion

This name is given to the transport of substances from a region of high concentration to one of low concentration, i.e. from within the intestinal lumen to the inside of the epithelial cell. Passive transport does not involve metabolic energy, and occurs in the same direction as the electrochemical gradient existing across the membrane. Carrier mechanism may or may not be involved. A simple example of passive transport is simple diffusion which occurs when a membrane is freely permeable to the solute molecules, and these move across the membrane from a region of high concentration to one of lower concentration. Another example of passive diffusion is solvent drag, in which
solute particles are carried along by the water stream.

(3) **Facilitated diffusion**

This is a special form of passive transport. In this process, substrates move in the direction of an electrochemical gradient, but their transport is facilitated by the existence of a carrier mechanism, and the rate of transport is greater than would be anticipated as the result of simple diffusion. Facilitated diffusion and active transport share certain features, both being carrier-mediated, and this distinguishes them from simple diffusion. These include selective transport of one substance as compared to another of similar molecules which can attach themselves to the same carrier, and saturation kinetics. The main difference between active transport and facilitated diffusion is that in the former the carrier is driven by metabolic energy, but not in the latter.

There is some argument about the relative importance of active transport and facilitated diffusion in the absorption of nutrients such as glucose and amino acids under normal conditions. In vivo, such substrates usually pass down a concentration gradient from the gut lumen into the blood stream, and it has been suggested that active transport may play little or no part in their normal absorption. However, mechanisms driven by metabolic energy cannot only move a substrate up a concentration gradient, they can also accelerate its movement down a gradient.

(b) **Amino acid absorption**

Amino acids are mainly absorbed by active transport. Höber and
Höber (1937) suggested that an active process was involved in amino acid absorption as they found the rate of absorption of glycine, valine and alanine were too fast to be accounted for without such a process.

Most subsequent work on amino acid absorption has been carried out on everted sac preparations of rat and hamster small intestine, first described by Wilson and Wiseman (1954). Some work has been carried out using the tissue-accumulation techniques of Finch and Hird (1960). These preparations can be used to measure the rise in the concentration of amino acids in the tissue water of the intestinal wall. If the ratio of this concentration to that in the fluid in contact with the mucosa is high, then there is evidence compatible with an active process. Agar et al (1953) confirmed and extended the study of amino acid with in vitro methods. In addition, they added an important improvement in methodology (Agar et al, 1954). They found, when tissue segments were incubated in solutions containing amino acids, that the tissue concentration of amino acid rose to a value considerably higher than that in the medium.

It is worth bearing in mind such considerations when one is showing the results of using in vitro isolated intestine. Attention must be paid to the perfusion medium which presents the environment of the internal pole of the mucosal cell, and it is through the fluid that the mucosa at rest gains its oxygen and nourishment.

Competition in the absorption of different amino acids has been demonstrated. When individual amino acids are introduced into the intestinal lumen separately, each amino acid is absorbed at a characteristic rate. However, these absorption rates vary with the
presence of other amino acids, and the fact indicates that amino acids compete with each other for their absorption (Pinsky and Geiger, 1952; Wiseman, 1955). Also, Christensen et al (1948) have published their results showing that when the concentration of various amino acids is increased in the plasma of intact guinea-pigs, there is evidence of competition for the cellular amino acid concentration mechanism.

Active transport has been shown to obey Michaelis-Menten kinetics (Smyth, 1967) and has been demonstrated using, among others, amino acids and glucose (Wiseman, 1954; Crane and Mandelstam, 1960). In these cases some degree of stereospecificity is evident since only the D-isomer of glucose will be transported (Wilson, 1962) and the L-form of the amino acid is greatly favoured over the D-form. Gibson and Wiseman (1951), and Elsden et al (1950) studied the rate of disappearance of both D- and L-isomers of thirteen different amino acids after a solution of the amino acid had been introduced into tied-off loops of rat small intestine in in vivo experiments. They showed that under the same conditions the L-isomer of the amino acid disappeared more rapidly than the D-isomer. They regarded this as evidence for an active process in amino acid absorption.

In vitro experiments have shown the same evidence (Schofield and Lewis, 1974; Clark, Gibson, Smyth and Wiseman, 1950; Matthews and Smyth, 1952, 1954; Wiseman, 1968), who all reported that L-amino acids were absorbed at a higher rate than corresponding D-isomers. The D-isomers are probably transported by passive diffusion. The chemical structure of an amino acid is important in determining its transport characteristics and the amino acids may be divided into several transport groups according to their structure. The members of each group appear to share the same transport mechanism but there is
competition for transport between members of different groups (Wiseman, 1955). However, no simple or rigid classification of these groups can be compiled, since the information bearing on transport characteristics comes from several species, and there may be other species with different transport characteristics (Matthews, 1968). Further, some amino acids may share more than one transport mechanism leading to cross-competition between members of groups originally thought to be distinct.

The mechanisms for the transport of amino acids and sugars are mutually inhibiting and sodium dependent (Reiser and Christiansen, 1969) and it is suggested that the transport may utilize a sodium pump mechanism. Also Wiseman (1977) reported that amino acids and dipeptides inhibit the active transport of D-glucose in the small intestine. He stated that the inhibition was not complete, and his finding is supported by that of Honegger and Semenza (1973) who showed that in hamster small intestine there is more than one transport system for D-glucose. Also, Fisher (1967) stated that amino acids can in turn inhibit the transfer of glucose across the intestinal wall to a notable extent.

Considering all investigators in this field who have looked at the range of competition, it seems that four groups of amino acids and related compounds have been recognized. These are as follows:

(1) Neutral amino acids (monoamino-monocarboxylic)

In this group are included the usual amino acids, glycine, alanine, valine, leucine, isoleucine, methionine, cysteine, threonine, phenylalanine, tyrosine, mono-iodotyrosine, tryptophan,
The neutral amino acids which probably share more than one transport mechanism, and overlapping specification, complicate the characterisation. For example, glycine and proline appear to be transported partly by the mechanism mainly responsible for the transport of the other neutral amino acids, but partly by some other special mechanism (Matthews, 1968). Newey and Smyth (1964) suggested that there may be sub-groups of neutral amino acids, each with a communal translocation mechanism. Matthews and Laster (1965) reported that competition between numerous pairs of amino acids corresponds on the whole to expectation based on the assumption that the respective half-saturation concentration ($K_m$) values represent measures of affinity for a common carrier. They conclude that the apparent lack of interaction found by other investigators is due to the wide spread of the $K_m$ value.

Wiseman's original studies indicated that this carrier would accept only amino acids with an unsubstituted amino group in the α position to the carboxy group with the exception of proline and hydroxyproline. Studies by Spencer et al. (1962); Wilson et al. (1960); and Lin et al. (1962) all strongly indicated that it would not accept amino acids with electrically charged side chains and that both the amino and the carboxylic acid groups were indispensable. However, it seems reasonable to conclude that these two groups are indispensable for transport by the carrier of neutral amino acids. From these studies it also appears that, as long as they do not give rise to a charged side chain, a number of different substituents are acceptable in the side chains of both aliphatic and cyclic amino acids (Wilson et al., 1960; Hagihira and Wilson, 1962; Hajjar and Curran, 1970).
Basic amino acids

This group includes arginine, cystine, histidine, lysine and ornithine. There is clear evidence that a number of neutral amino acids are transported by the carrier of basic amino acids. The proof that the basic amino acids are actively transported was presented by Hagihira et al (1961). These data, together with those of Robinson and Felber (1964); Robinson (1966, 1968); Münck (1966), and Chez et al (1971) suffice to group together arginine, cystine, histidine, lysine and ornithine. Therefore it can be stated that the basic amino acids are the primary users of the carrier.

The main stimulus of continued interest in intestinal transport of basic amino acids, and its relation to that of the neutral amino acids, has probably come from the observed ability of some neutral amino acids to stimulate transepithelial transport of lysine (Münck, 1965; 1966; Münck and Schultz, 1969) and to apparently stimulate a stable state of epithelial accumulation of lysine and arginine (Robinson and Felber, 1964; 1965; Robinson, 1968), and finally from the trans-stimulation by leucine of lysine influx across the brush border membrane of rabbit ileum (Münck and Schultz, 1969). These interactions have subsequently been successfully studied by Reiser and Christiansen (1969) with respect to types and specificities of interactions, and with respect to the role of sodium. Evidence that the transport of neutral and basic amino acids is mediated by transport mechanisms with overlapping specificities for these two groups of amino acids has been presented for the small intestine (Robinson and Felber, 1965; Münck, 1966). In most instances, the evidence for interactions between basic and neutral amino acids consists of the
demonstration that the transport of an amino acid from one group is inhibited by an amino acid from the other group and vice versa. However, Robinson and Felber (1965), and Munck (1966), have reported an interaction between basic and neutral amino acids in the small intestine that is characterized by the ability of the neutral amino acid to stimulate the transport of the basic amino acid. Thus, Munck (1966) has shown that the rate of lysine transport across everted sacs of rat ileum is markedly enhanced by the presence of a low concentration in the mucosal medium.

**TABLE 1.8** A comparison between neutral and basic amino acid influx in the L-form K expressed in mM for influx of amino acids across the brush border membrane of rabbit ileum and rat jejunum

<table>
<thead>
<tr>
<th></th>
<th>Neutral$_1$</th>
<th>Neutral$_2$</th>
<th>Basic$_3$</th>
<th>Neutral$_4$</th>
<th>Basic$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>9</td>
<td>10.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>7</td>
<td>4.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>6</td>
<td>4.8</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td></td>
<td>1.3</td>
<td>3</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.5</td>
<td>2.7</td>
<td>8</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td></td>
<td>4.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>6</td>
<td>2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td></td>
<td>10</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

3 - Munck & Schultz (1969) 4 - Munck & Rasmussen (1975)
Finally, Ralph (1978) reported that there seems to be one transport mechanism for neutral amino acids, and another mechanism may be responsible for the transport of basic amino acids.

(3) **Acidic amino acid (α-amino-dicarboxylic acids)**

Because of extensive transamination of aspartic and glutamic acids by intestinal tissues (Neame and Wiseman, 1956, 1957; Ramaswamy and Radhakrishnan, 1970) the transport characteristics for these amino acids can only be defined for unidirectional influx across the brush border membrane. Very little work has been done on this function and only Schultz *et al* (1970) have studied it. Ralph (1978) reported that glutamic acid and aspartic acid may diffuse passively through the intestinal wall. The question of mutual inhibition between these amino acids and neutral and basic amino acids was not examined. Thus, it is presently not known whether or not aspartic acid and glutamic acid are transferred across the brush border membrane by a carrier of their own.

(4) **Imino acid**

In this group are found proline, hydroxyproline and sarcosine N-, mono-, di- and trimethylglycine. The group was shown by Hagihira *et al* (1962) to be transported in hamster small intestine by a separate mechanism. It is to be noted that imino acids are present as peptides, in a proportion of 90% till their absorption (Nixon and Mawer, 1970a, 1970b).
Sodium has been shown to influence the rate of transport of many solutes in a wide variety of animal cells (Riklis and Quastel, 1958; Christensen and Antonioli, 1969; Christensen and Handlogten, 1969). This sodium dependence also appears to be closely related to the ability of these cells to transport the other solutes against an electrochemical potential difference. This sodium ion dependent transport process has considerable physiological importance and is being investigated in this work. Numerous sodium-dependent transport processes have been subjected to detailed kinetic analysis. The evidence now available suggests that these systems may represent one of the most interesting examples of the coupling of transport processes yet observed in biological membranes. The possibility that the energy for these processes is derived from the asymmetric distribution of sodium ion across cell membranes may provide additional insight into the physiological function of the active transport systems characteristic of all animal cells. Parrish and Kipnis (1964, 1965) provide a good demonstration of the fact that it is not the tissue but the nature of the transport process that determines the involvement of sodium ions. Kleinzeller et al (1967) showed that the uptake of glucose or galactose was inhibited by removal of sodium ions from the external medium. Schultz et al (1966) demonstrated this point by some observations on alanine transport by rabbit ileum (Fig. 1.7). When the incubation medium contains 140 mM sodium, the cells rapidly accumulate alanine in a chemically unaltered, osmotically active form, and the final steady-state intracellular concentration exceeds significantly that in the external medium. Also they observed an increase in cell water of mucosal cells as alanine was accumulated.
FIG. 1.7 Time course of L-alanine uptake. All tissues were from the same animal and were preincubated for 20 minutes before addition of 5 mM L-alanine. Medium was normal buffer (after Schultz et al, 1966).
There are at least two explanations for the sodium dependence of amino acid absorption. The first is that sodium is a co-substrate for the transport process, so that the movement of sodium and the involved solute are coupled in some manner. The second is that sodium influences solute transport indirectly and its flow is not coupled to the solute flow. For example, sodium could bring about changes in membrane structure that make the transport site more accessible to the solute or could facilitate a direct coupling between the transport system and a source of metabolic energy. Further evidence suggesting coupling between the flows of organic solutes and sodium was provided by the observation that addition of actively-transported amino acids or sugar to the solution bathing the mucosal surface of small intestine resulted in an increase in transmucosal electrical potential difference, short circuit current, and in some instances net sodium transport from mucosa to serosa.

The important role of the sodium ion in the active transport of certain nonelectrolytes such as sugar and amino acids has been demonstrated by many research techniques (Csaky, 1961, 1963; Bihler and Crane, 1962; Crane et al, 1965; Schultz et al, 1967). However, much of this demonstration effort has been directed at renal (Kleinzeller and Kotyk, 1961; Fox et al, 1964; Hauser, 1969) and intestinal tissue (Bihler and Crane, 1962, Csaky, 1963; Crane et al, 1965; Schultz et al, 1967). Factors that may affect the process of active transport include the high degree of dependence on sodium ions for accumulation of metabolites against a concentration gradient (Csaky, 1961, 1963; Kotyk, 1961; Fox et al, 1964; Crane et al, 1965; Goodman, 1966; Inui and Christensen, 1966; Schultz et al, 1967; Holdsworth and Wilson, 1967; Wheeler and Christensen, 1967; Hauser,
Fig. 1.8 taken from Schultz (1977) shows a model for carrier-mediated processes at the mucosal membrane that bring about the coupled entry of sugar or amino acids related to Na$^+$ into the epithelial cell. Also the model illustrates an energy-dependent, ouabain-sensitive mechanism at the basolateral membrane capable of actively excluding Na$^+$ from the cell. It was suggested by Schultz and Zalusky (1964) that this pump maintains a low intracellular Na$^+$ concentration and thereby provides a driving power for the coupled entry step, and energizes the 'uphill' movement of the amino acid or sugar. The movement 'uphill' of the non-electrolytes need not be directly coupled to a source of metabolic energy; instead, all of the required energy could be derived from the Na$^+$ gradient across the mucosal membrane which is established and maintained by the basolateral Na$^+$ pump. The location of this pump was initially supported by the finding that ouabain only inhibits active Na$^+$ transport when present in the serosal solution. The presence of this glycoside in the mucosal solution alone is ineffective (Schultz and Zalusky, 1964). The later investigations by Quigley and Gotterer (1969); Douglas et al, (1972); Fujita et al (1972) were concerned with the distribution of enzyme activities in the mucosal and basolateral membranes of fragmented intestinal cells and autoradiographic studies of [3H] ouabain binding. Stirling (1972) has demonstrated clearly that the ouabain-sensitive, Na$^+$-K-ATPase is localized almost exclusively in the basolateral membranes.

(d) **The effect of antibiotics on growth and intestinal absorption in the rat and chick**

It was Vuillemin (1889) who first used the word 'antibiosis' to describe a type of association among living systems in which one
FIG. 1.8 Model for interaction between transcellular sugar (S) and Na transport (from Schultz, 1977)
organism destroys another in order to sustain its own life. Papacostas
and Gate Ltd described the meaning of the word by differentiating the
injurious effect of one organism upon another in vitro, a type of
association called antibiosis, from the same effect occurring in vivo,
an association called antagonism. The word 'antibiotic' with new
meaning, was suggested by Wakesman (1947) to designate a chemical
substance of microbial origin which had the property to inhibit the
growth of microorganisms, namely, "An antibiotic is a chemical
substance, produced by microorganisms, which has the capacity to
inhibit the growth and even to destroy bacteria and other micro-
organisms".

Several attempts have been made to broaden the word 'antibiotic'
to include substances produced by higher plants and animals that
possess antimicrobial properties. The definition thus becomes:
"Antibiotics are chemical substances that are produced by living
organisms and have the capacity to inhibit the growth of micro-
organisms or other living cells".

The fact that antibiotics stimulate the growth rate of chicks
was discovered by chance by Moore et al (1946) when they found that
sulphasuxidine and streptomycin improved the growth of chicks fed on
a purified diet. The reason for their study was to sterilize the
gastrointestinal tract in order to evaluate the role of its flora in
providing the chick with essential nutrients. Aureomycin and strepto-
mycin were found to be excellent substitutes, but it was also found
that the growth-promoting activity of the material was greater than an
equivalent amount of pure vitamin B_{12} (Stokstad et al, 1949; McGinnis
et al, 1949). Stokstad and Jukes (1950) showed that pure aureomycin
would stimulate the growth of chicks in the presence of vitamin B$_{12}$.

Patric (1951) reported that antibiotic growth stimulation was variable and unpredictable (Scott and Glista, 1950; Damron and Harms, 1971). They found no response on body weight or feed efficiency when aureomycin and terramycin respectively were offered to their chicks. Heuser and Norris (1962) commented that the growth response was variable even with the same antibiotic in different experiments, while Marusich et al (1974) showed that penicillin in turkeys resulted in increased growth and improved feed conversion. However, the amount of antibiotic added to the diet played a predictable role, because by this time the antibiotics were in known and more active forms. Orr et al (1974) demonstrated in several experiments that addition of 2.6 ppm penicillin to the medicated feed significantly increases weight but not the food efficiency. Addition of 55 ppm penicillin in the same condition showed significant differences in weight gains and feed efficiency. Graber et al (1974) also reported the response of broiler chicks to several antibiotics, each at 50 ppm. Body gains of 152, 190, 230 and 227 grams resulted from a control diet without antibiotics, and the same diet with chlorotetracycline, penicillin and zinc bacitracin respectively. The latter is not used in human medicine and for this reason is now favoured in animal feeds.

There is little evidence concerning the relationship between intestinal absorption and dietary composition. Eyssen and De Somer reported in a series of studies (1963a, 1963b, 1965, 1967) that in certain conditions chicks excrete less fat when fed a diet based on sucrose. It was found that antibiotic supplementation to the chicks' diet affected the growth response (Scott et al, 1951). The growth
response to antibiotics was obtained on all-vegetable protein diets rather than those rations containing animal protein, and there is a suggestion that antibiotics functioned by sparing a growth factor associated with a protein from an animal source (Heuser and Norris, 1952; Braude et al, 1953). Also, Stokstad et al (1953) showed that aureomycin gave a favourable response to growth rate, using a diet containing sucrose as the main carbohydrate source; however, the response was absent if starch was used instead of sucrose.

There are a number of antibiotics which promote chick growth but are not readily absorbed by the gut. It was suggested that they showed their effect by acting on certain components of the intestinal flora. This hypothesis can be proved by the lack of effect of antibiotics on the growth of germ-free animals. The effect of antibiotics on growth rate can be explained in a number of ways: (i) stimulation of organisms which supply it with growth factors; (ii) the elimination or suppression of organisms which compete with the host for nutrients; (iii) reduction of toxigens. However, it has been demonstrated that the variation in the effects of the antibiotics on the intestinal flora has been great and similar to the variation in the result of antibiotic growth response itself. On the other hand, Sieburth et al (1954) reported that the small intestine may be the site of microbial growth-depressing activity and that the maximum response to the antibiotic is usually shown during the first fourteen days of age in chicks (Heuser and Norris, 1952).

It has been well reported that feeding antibiotics improves the efficiency with which the chick utilizes its food (Davis and Briggs, 1951; Morrison et al, 1954). Rosenberg et al (1952) reported that
with chicks there is a significant growth response to terramycin, but this does not apply to the feed efficiency. This observation is in agreement with Slinger et al (1954) who found that there was no improvement on food efficiency conversion when antibiotics were added. It has been reported that chicks fed with antibiotics develop a greater appetite than those which are not. This may explain the antibiotic growth response, since increasing the intake of nutrients causes an increase in the growth rate (Scott and Glista, 1950; Brown et al, 1952; Dymsza et al, 1953, Slinger et al, 1954).

It was suggested that antibiotics improve protein utilization (McGinnis, 1951; Slinger et al, 1952; Baldini et al, 1953; West and Hill, 1957). There is little evidence that antibiotics spare individual amino acids. Jones and Combs (1951) reported that no sparing effect on lysine or methionine could be demonstrated.

Growth promotion for broilers resulting from addition of antibiotics to their feed is generally accepted. However, apart from disease control, there is a limited amount of data on the effect of water soluble antibiotics on the performance of apparently healthy broilers. Kolar and Seymour (1971) reported that broilers receiving spectinomycin with drinking water became significantly heavier than controls. The same workers showed significant differences in food conversion ratio between groups receiving spectinomycin and those which did not.

A structural change in a metabolite may yield a product which no longer functions normally in metabolism, and which inhibits the metabolism of the normal analogue. The antagonist might therefore in some way inhibit the metabolism of its natural analogue and produce
effects comparable to a deficiency of the natural metabolite. It was found by Dyer (1938) that ethionine did not substitute for methionine in supporting the growth of rats. She also observed that rats fed with ethionine lost weight more rapidly than another group on a methionine-free diet. It has been found that ethionine inhibits the growth of microorganisms (Roblin et al, 1945; Halverson and Spiegelman, 1952). Simpson et al (1950) found that ethionine inhibits the incorporation of methionine sulphur and of glycine into the proteins of rats, and that it also inhibits the conversion of methionine to cysteine. Although there is considerable information and research concerning mechanisms that regulate intestinal amino acid absorption in chicks, very little is known about actinomycin D. Ferraro et al (1976) showed that actinomycin D caused a significant diminution of the intestinal absorption of phosphate when he studied rats in vivo. The proteolytic enzyme, pepsin, is secreted by the mammalian stomach and in the proventriculus of chicks. It is known that this enzyme cleaves the amino acid residues at leucine, isoleucine and tryptophan. Trypsin, secreted in the small intestine, cleaves at lysine and arginine residues. It was also suggested that the two major proteolytic enzymes, pepsin and trypsin, and others, secreted in both stomach and intestine of the rat and chick, do not digest the molecules of actinomycin D and puromycin. This would not, therefore, stop their action of preventing biosynthesis in the gut and flora of rats and chicks.
D. OBJECT OF THE INVESTIGATIONS

It is evident from the preceding review that the amino acid composition of the diet plays an important role in the growth and development of the commercial broiler chick. Since these requirements change with age it seems necessary to have a reliable basis on which changes in the diet can be formulated. In the past, most studies on this problem have been based on the determination of the growth response and food utilization of the birds. However, in order that dietary amino acids can be made available for growth they must be absorbed from the intestine. It is well known that in mammals the absorption rate depends on a number of factors and differs for individual amino acids. Furthermore, amino acid absorption is known to change with age. The present study was undertaken to examine some of these factors in the developing chick using an in vitro technique. Thus the rate of amino acid intestinal absorption and concentration gradient related to age and the effect of glucose is considered. In addition, the effect of the antibiotics actinomycin D, oxytetracycline, penicillin and puromycin is studied in relation to its effect on the absorption of L-methionine. It was hoped that the results determined by this method could be related to requirements.

Lysine and sulphur amino acids have a particular relevance to the requirements of broiler chicks. Lysine, methionine and cysteine are known to be key factors in broiler feeding for their direct effect on body weight, food utilization, feather development, general health and cost of production. Therefore they are chosen as the main theme of this investigation.
The replacement of cysteine in broiler feeding diets by methionine during growth could explain some feeding problems. Some of the workers quote the requirement of each of the two sulphur amino acids separately, others present the requirement as a combination of both. It was considered worthwhile performing studies of the absorption characteristics of each of these sulphur amino acids.

The upper part of the small intestine is the principle region of the alimentary tract where amino acid absorption takes place. The upper jejunum of rats, representing the mammalian species, and chicks, representing the avian species, were investigated. The effect of antibiotics given orally on the rate of amino acid absorption was investigated as these drugs have been reported to help in preparing the interior of the gut for more efficient absorption of amino acids and other nutrients. More than one kind of antibiotic was used in order to establish whether or not these have similar effects on amino acid absorption in broiler chicks.
CHAPTER 2

MATERIALS AND METHODS
A. EVERTED GUT SAC TECHNIQUE

(a) Chemicals, reagents and apparatus

All reagents used were 'Analytical Reagent' (A.R.) Grade except where stated otherwise. Aqueous solutions were made up in glass distilled water.

All chemicals were supplied by British Drug Houses Ltd (B.D.H.), Poole, Dorset, and Sigma London Chemical Co Ltd., Fancy Road, Poole, Dorset.

The 95% oxygen, 5% carbon dioxide gas mixture was supplied by British Oxygen Co Ltd., Medical Gases Division.

The absorption produced during amino acid assays was read on a Cecil SE 272 ultraviolet spectrophotometer (Cecil Instruments Ltd., Milton Trading Estate, Cambridge Road, Milton, Cambridge).

(b) Animals, accommodation and diets

(1) Chicks

Male broiler chicks, Ross 1 day old, were supplied in batches of 80 chicks by Ross Poultry, The Broadway, Woodhall Spa, Lincolnshire. They were kept in an electrically heated battery cage measuring 2 x 1 m, equipped with a heat control thermostat. The floor consisted of a wire screen. Food and water were offered ad libitum. The cage contained four drinking troughs and two feeding troughs, both of which were filled daily and as required.

The room in which the chicks were kept was windowless and measured
3.5 x 2.5 m. A conventional ventilation system was present and air was extracted via a fan. There were four settings by which the ventilation could be used: 1/4, 1/2, 3/4 and full. It was used on 1/2 for the first ten days of the experiment, and full from ten days onwards. Droppings were collected daily from three trays placed beneath each cage.

The lighting system consisted of three red 40W fluorescent tubes. The temperature was controlled and maintained between 24–32°C according to the age of the chicks. The higher temperature was used for day-old chicks and this was progressively reduced as the chicks grew.

A commercial diet was used to rear the chicks. This was supplied by Wm Lillico and Son Ltd., Wonham Mill, Betchworth, Surrey. Details of the diet are shown in Table 2.1. The diet contained satisfactory levels of crude protein, metabolisable energy and the amino acid requirements of chicks from hatching to 7 weeks of age (Table 2.1). With the exception of the feeding experiments, chicks were reared throughout on this diet. They were selected according to age and body weight as required for each part of the study. Details of the number of chicks used for each experiment are given in Section E. "GROWTH EXPERIMENT!".

**TABLE 2.1**

Chemical composition of mixture used for broiler diet

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>22</td>
</tr>
<tr>
<td>Fat</td>
<td>2.5</td>
</tr>
<tr>
<td>Fibre</td>
<td>4.0</td>
</tr>
<tr>
<td>Metabolisable energy</td>
<td>2990 Kcal/kg</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.86</td>
</tr>
</tbody>
</table>
Rats

In order to standardise the conditions of feeding for each experiment, food was removed from the cages 24 hours before killing.

Weanling and adult Wistar albino rats of an SPF derived colony (University of Surrey) were used in all experiments conducted. Food and water were given ad libitum. Food was supplied by Spratts laboratory diet No. 1 (expanded breeding diet), and its composition is shown in Table 2.2.

### TABLE 2.2

Percentage chemical composition of the feed mixture used for rats

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>g/100 g of diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>21.5</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>2.7</td>
</tr>
<tr>
<td>Fat</td>
<td>4.5</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.1</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.39</td>
</tr>
</tbody>
</table>

The rats were caged in plastic cages with wire mesh floors and lids which prevented them from coprophagy and eating the wood shavings during the starvation period prior to killing. Water was offered at all times, including during starvation.

(c) Everted gut sac preparation

All the animals were anaesthetised with ether for about two minutes then killed by cervical fracture. The abdomen and thorax were opened by a midline incision. The sacs were prepared according to the
method first described by Wilson and Wiseman (1954a), and in greater detail by Wiseman (1961).

Several workers have demonstrated that different intestinal regions show different levels of transport activity (Barry et al., 1961; Hindmarsh et al., 1967). In order to specialise this investigation, a defined and reproducible region of the small intestine was selected, i.e. the upper part or first 20 cm of the small intestine in both rats and chicks (Figs. 2.1 and 2.2). Five animals were chosen for each experiment and two gut sac samples were taken from each animal. Thus each experiment contained ten different samples.

The perfusion medium was Krebs and Henseleit buffer (1932). Table 2.3 shows the composition of the perfusion medium which was used throughout all the experiments concerning intestinal absorption.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>ml volume</th>
<th>mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl</td>
<td>(0.154 M)</td>
<td>100</td>
</tr>
<tr>
<td>1.15% KCl</td>
<td>(0.154 M)</td>
<td>4</td>
</tr>
<tr>
<td>2.11% KH₂PO₄</td>
<td>(0.154 M)</td>
<td>1</td>
</tr>
<tr>
<td>3.82% MgSO₄</td>
<td>(0.154 M)</td>
<td>1</td>
</tr>
<tr>
<td>1.3% NaHCO₃</td>
<td>(0.154 M)</td>
<td>21</td>
</tr>
</tbody>
</table>

In general, glucose was added to provide a 0.5% solution. Each batch of medium was about 10 litres, this being sufficient for one full experiment. During the course of the experiment, which lasted
FIG. 2.1 Abdominal parts of the alimentary canal and associated structures (displayed). (After Rowett, 1957).
FIG. 2.2 Digestive tract of the chicken.

1 and 2, oesophagus and crop; 3, proventriculus; 4, liver; 5, hepatic duct; 6, gall bladder; 8, gizzard; 9, duodenum; 10, pancreatic ducts from dorsal lobe; 11, pancreatic ducts from ventral lobe; 12, dorsal lobe of pancreas; 13, ventral lobe of pancreas; 14, upper and lower segments of small intestines; 15, ceca; 16, large intestine or rectum; 17, cloaca.

(After Sturkie, 1965).
between 3 and 5 weeks, the medium was kept in the cold room at $4^\circ C$. Just before use the perfusion fluid was gassed at $37^\circ C$ for the rat experiment and $41^\circ C$ for the chick experiment, and bubbled with 95% $O_2$/5% $CO_2$ for 20 minutes; the final pH of this mixture was 7.2-7.3.

The intestine was sectioned at the region of the upper duodenum and at the ileocaecal junction, removed, and the lumen was cleaned of its contents by allowing 'oxygenated' perfusion medium, Krebs and Henseleit (1932), gassed with 95% $O_2$/5% $CO_2$ at $37^\circ C$ in the case of rats or at $39^\circ C$ in the case of chicks, to stream through the intestine as shown in Fig. 2.3(a). The hydrostatic pressure of the perfusion medium was not allowed to exceed 50-60 cm because with higher pressures damage may occur to the intestine by excessive dilation.

Subsequent manipulations were performed quickly at $0^\circ C$ with minimal handling. Wiseman (1961) quotes 3 minutes as an adequate time from killing the animal to completion of eversion. All acute bends which may obstruct the passage of the intestinal contents were straightened and finally a length of 15-20 cm was taken from the upper duodenum. A glass rod of 4 mm diameter with a smoothed bulbous end was used for the eversion of the intestine (Fig. 2.3b). A cotton ligature was used to secure the intestine on the indentation in the rod. The intestine was then rolled gently over its invaginated end until the complete length of gut was impaled on the rod. One cm of the distal end of the intestine was not everted to differentiate between the two samples taken.
FIG. 2.3 Stages of gut sac eversion
(d) Experimental procedure for intestinal transport studies

A length of 5-7 cm was taken for each sample of the intestine; the younger the animal the longer the sample, since the diameter is less in the younger animal. This provides the same volume in all experiments and enough space for the solution inside the intestine. This gives similar pressure within the gut in all experiments.

One end of the sample was tied off with a thread ligature. The ligature was left for easy handling and a definite length of the sac was chosen (Fig. 2.3c). A blunt needle, attached to a 1 ml syringe was introduced into the intestinal lumen and a second ligature was left loosely around the blunt needle. The amount of amino acid perfusion medium (equilibrated with 95% O₂/5% CO₂) mixture was 0.5 ml in all experiments. The concentration of all amino acids used was 0.8 mM/litre inside the sac and in the flask. This was prepared from 0.1 M stock solution kept in 10 ml test tubes and well frozen at -15°C. After that amount was injected the ligature was tightened as the sample was slowly withdrawn and finally tied off (Fig. 2.4a).

Two sacs from each animal were immersed in a 75 ml flask containing 40 ml of outer (mucosal) fluid, i.e. the same concentration of amino acid solution was used for distending the sac, prewarmed either to 37°C in the case of rats, or to 41°C in the case of chicks. This volume was sufficient to permit adequate changing of its surface and overall mixing when the flask was gently shaken. The shaking was carried out in a water bath at the appropriate temperature for the time requested, gassing with 95% O₂/5% CO₂ throughout the incubation period (Fig. 2.4b).

It was found, in practising the technique, that rapid shaking was seen to cause shedding of the mucosa so that the shaking rate of 80 oscillations/minute recommended by Wiseman (1961) was reduced to
FIG. 2.4 The injection and incubation of gut sac samples
35 oscillations/minute (amplitude 5 cm) to minimise the degree of damage to the mucosa during this period.

At the end of the incubation period, the sacs were removed with forceps and a short length of thread ligature was left at one end of the sac, and washed by dipping through cold ice perfusion medium at 0°C. This was gently blotted with smooth, hardened filter paper and drained into small test tubes (1 ml size). The serosal fluid was drained to give the final serosal fluid. A sample of the flask fluid (final mucosal fluid) was also retained for ninhydrin determination of the amino acids present (Lee and Takahashi, 1966).

Wiseman (1955) indicated that this method recovers about 96% of the serosal fluid. Any sacs whose serosal fluid volume had decreased during incubation were discarded.

The ligature was cut off as close as possible to the point of tie from both ends and put into a 2 ml test tube and weighed to find the effective weight of 'fresh' tissue. The sacs were placed in an oven at 105°C for 24 hours, removed and placed in a small desiccator to cool for one hour. The 'dry' weight of the tissues was recorded.

B. DEFAITING OF INTESTINAL TISSUES

The dry sac was kept in the same 2 ml test tube throughout each experiment. Diethyl ether was used to extract the fat from the dry intestinal tissues. About 2 ml diethyl ether was added to each sample twice and shaken for ½ hour the first time, emptied, refilled, and again shaken for 10 minutes before decanting the ether. At both
times samples were shaken on a revolving circular board of 24 in diameter and rotated at 40 oscillations/minute. Samples were placed in the oven for 30 minutes, cooled for one hour and reweighed. The weight of the dried, defatted tissues was recorded. Table 2.4 shows the method by which the weight of the sac was established:

TABLE 2.4 Measurements of the dry defatted sac

(a) Weight of test tube, empty.
(b) Weight of test tube + fresh sac.
(c) Weight of test tube + dry sac.
(d) Weight of test tube + dry defatted sac.

(b)-(a) = Weight of fresh sac.
(c)-(a) = Weight of dry sac.
(d)-(a) = Weight of dry defatted sac.

The dry defatted sac was used for the rate of absorption in all experiments concerned.

C. DETERMINATION OF AMINO ACIDS

(a) Ninhydrin amino acid reaction

The quantitative determination of amino acids has been discussed by many workers, including Meister (1965). The procedure employed here involved the development of a chromogen by heating the amino acid with ninhydrin. The reaction of amino acids with ninhydrin is of considerable value in the detection of amino acids in their quantitative
determination. Most of the amino acids react specifically with ninhydrin to yield hydridantin, carbon dioxide, ammonia, and the corresponding aldehyde (Van Slyke et al, 1941, 1943; MacFadyen, 1944; Moore and Stein, 1948).

\[
\text{NH}_2 + \text{RCHCOOH} \rightarrow \text{RCHO} + \text{CO}_2 + \text{NH}_3
\]

The majority of α and β or γ-amino acids produce colours in the purple range with an absorption maximum in the region of 570 nm. Studies of amino acid measurement have been carried out by many workers such as Moore and Stein (1948); Troll and Cannan (1953); Moore and Stein (1954); Yemm and Cocking (1955); Kalant (1956); Rosen (1957); Matheson et al (1961); Matheson and Tattrie (1964). It was important to have available a suitable quantitative method for the determination of the concentration of amino acids throughout all experiments. For this purpose, the method should be sufficiently general to include the determination of all amino acids used. In addition, the method should be as sensitive as possible to allow the determination of low concentrations of amino acids present.

It is also advantageous if the laboratory procedure is fairly
simple, so allowing determinations to be carried out on large numbers of effluent samples. During studies of amino acid metabolism and protein biosynthesis, attempts were made by Lee and Takahashi (1966) to develop a simpler, more sensitive and more specific assay of \( \alpha \)-amino nitrogen for practical use. These workers reported that the procedure they used had several advantages compared with those previously reported. Glycerol, which is stable and non-volatile, is a better medium for colour development. The reaction does not require other additional compounds to facilitate colour yield. The reagent was stable even after mixing, whereas reagents reported previously could not be mixed together before use because of instability. This method does not require dilution after heating, described by Yemm and Cocking (1955); Rosen (1957); Matheson and Tattrie (1964); Kalant (1965). Colour development is not affected by the common protein precipitants, which is an important reason for using this method. In addition, the colour is quite stable. Sensitivity is high and the reproducibility of the colour development was good (see Table 2.5 and Fig.2.5).

<table>
<thead>
<tr>
<th>mM/lit conc</th>
<th>Mean of O.D.-Blank</th>
<th>SiE.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>0.101 *</td>
<td>0.0023 +</td>
</tr>
<tr>
<td>0.8</td>
<td>0.203 *</td>
<td>0.0028 +</td>
</tr>
<tr>
<td>1.2</td>
<td>0.304 *</td>
<td>0.0046 +</td>
</tr>
<tr>
<td>1.6</td>
<td>0.407 *</td>
<td>0.0017 +</td>
</tr>
</tbody>
</table>

* Each of the four figures presented are the mean of 10 readings.
FIG. 2.5  L-alanine standard graph

Optical density at 570 nm
Finally, this method could be used to determine 0.005-0.2 μmole of amino acid in 0.1 ml. For the reasons given above, it was decided that the method of Lee and Takahashi (1966) was most suited for these experiments and was therefore used.

(b) **Preparation of buffers and reagents**

Citrate buffer solution:

A. 0.5 M solution of citric acid M.W. 210.4

B. 0.5 M solution of sodium citrate M.W. 294.10 \( \text{C}_6\text{H}_5\text{O}_7\text{Na}_3\cdot2\text{H}_2\text{O} \)

Solution A was added to solution B to give the pH of 5.5 required. The reagents were mixed in large quantities to cover each experiment in order to make the comparisons of different results as close as possible. Table 2.6 below shows the chemical composition of the reagent.

**TABLE 2.6** The chemical composition of the reagent used for amino acid determination

<table>
<thead>
<tr>
<th>Volume</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 ml</td>
<td>0.5 M citrate buffer solution (pH 5.5) with 1% ninhydrin (2 g)</td>
</tr>
<tr>
<td>480 ml</td>
<td>Glycerol</td>
</tr>
<tr>
<td>80 ml</td>
<td>0.5 M citrate buffer solution (pH 5.5)</td>
</tr>
</tbody>
</table>

These solutions added together give a solution of 760 ml final reagent mixture (pH 6.0).

(c) **Procedure of amino acid determination**

All the sac contents were diluted 10 times by adding 0.1 ml of the
contents to 0.9 ml perfusion medium. A blank and four different concentrations of amino acids, i.e. 0.4, 0.8, 1.2 and 1.6 mM/litre were prepared in advance. 0.1 ml of the diluted intestinal serosal fluid was taken from each of the ten samples in duplicate. Also two 0.1 ml samples were taken from the flask content mucosal fluid to confirm that the same concentration was used (i.e. 0.8 mM/litre). The samples were taken after mixing all the flask contents together. The volume of 0.1 ml was taken from the blank and the four different concentrations in duplicate. All of the 0.1 ml samples were put into 5 ml glass test tubes by using a 100 µl pipette. They were mixed with 1.9 ml of a ninhydrin-citrate-glycerol reagent mixture dispensed by an Oxford pipettor. The reagent consisted of 0.5 ml of 1% ninhydrin solution in 0.5 M citrate buffer (pH 5.5), 1.2 ml glycerol and 0.2 ml of 0.5 M citrate buffer solution (pH 5.5) which were made up in 760 ml quantities prior to analysis.

All the tubes were heated in a boiling water bath for 12 minutes and cooled in a water bath at room temperature for 5 minutes. The duplicate samples were shaken for 5 seconds by a vortex mixer and read at 570 µm within 1 hour. The blank and the four amino acid standard solution samples were run at the same time and yielded the correct standard curve, proving the method suitable for the experiments to be carried out.

D. HISTOLOGY OF THE SMALL INTESTINE

(a) Preparation of paraffin wax-embedded sections staining with haematoxylin and eosin
(1) **Fixation**

Pieces of intestine (2-3 cm length) were fixed in 10% neutral, buffered formalin for 4-5 days. The formula of the buffered formalin was as follows:

- **Formalin** 100 ml
- Sodium dihydrogen orthophosphate $\text{NaH}_2\text{PO}_4\cdot 2\text{H}_2\text{O}$ 4.5 g
- Anhydrous disodium hydrogen orthophosphate $\text{Na}_2\text{HPO}_4$ 6.5 g

Distilled water was added to 1 litre.

(2) **Processing**

A piece of intestine (2-3 mm length) was cut from each sample and placed in a metal processing container. The containers were placed in a tissue basket which was then fitted to the Histokinette automatic tissue processor. Tissues were transferred automatically from one beaker of fluid to the next and the processes of dehydration, clearing and impregnation with wax were carried out using the following processing schedule.

(3) **Dehydration**

The sample was placed in the following solutions respectively:

- 70% alcohol 1 hour
- 85% alcohol 1 hour
- 95% alcohol 1 hour
- 100% alcohol I 1 hour
- 100% alcohol II 1 hour
- 100% alcohol III 1 hour
(4) Clearing and impregnation

The sample was washed out with toluene kept in two different containers for 1 hour, transferred into pure paraffin solution and kept at 58°C for one hour. It was also kept in a fresh solution and condition but different container, but again for a period of 1 hour.

After this process the tissue containers were transferred to a vacuum-embedding oven containing pure paraffin wax (melting point 56°C) at a temperature of 58°C for half an hour. This acted as a third wax bath and the reduced pressure aided impregnation by ensuring that any remaining air bubbles and clearing agent were removed.

The tissue was 'blocked' by removing it from the container using a pair of electrically heated forceps and placing it in a plastic mould filled with melting wax. The tissue was positioned so that the surface to be cut rested on the base of the mould. A plastic block was placed in the mould, together with the sample number label for each sample. The wax was then allowed to cool before the wax block was pressed out of the mould.

(5) Section cutting

Sections were cut from the blocks at a thickness of 6 µ using an American Optical Spencer 820 rotary microtome. These were floated on distilled water at a temperature of 50°C to remove creases and flatten them. These sections were then mounted on slides by half submerging a clean slide into the water near them and withdrawing it, so bringing the flattened section with it.

Slides were placed initially on a drying hot plate and then left in an incubator at a temperature of 37°C overnight to dry.
(6) Staining

The sections were stained with Ehrlich's acid haematoxylin and eosin using the following method. Slides were treated as follows:

(i) Xylene to remove wax. 2 minutes
(ii) Absolute alcohol 1 minute
(iii) 70% alcohol 1 minute
(iv) 50% alcohol 1 minute
(v) Rinsed with distilled water 1 minute
(vi) Stained in Ehrlich's acid haematoxylin 15 minutes
(vii) Blued in tap water 5 minutes
(viii) Differentiated in acid alcohol (1% hydrochloric acid in 70% alcohol) by agitating for about 5 seconds
(ix) Returned under tap water until colour becomes blue 1-2 minutes
(x) Examined under a low power microscope to ensure sections sufficiently differentiated
(xi) Section again washed with water until blue 15 minutes
(xii) Counterstained in 1% aqueous eosin 2 minutes
(xiii) Washed under water tap to differentiate eosin 2 minutes
(xiv) Dehydrated in 85% alcohol ½ minute
(xv) Dehydrated in 100% alcohol ½ minute
(xvi) Dehydrated in 100% alcohol ½ minute
(xvii) Cleared in xylene ½ minute
(xviii) Cleared in xylene ½ minute

The sections were mounted in D.P.X. (British Drug Houses Ltd) by placing a drop of the mountant on a clean coverslip, taking the slide directly from the xylene, inverting it over the coverslip, and pressing gently so that the mountant spread under the coverslip.

Slides were examined at a magnification of ×40, ×100, and ×400
using a Vickers M15C microscope.

Photographs of the sections were taken by a Vickers M15C microscope connected to an automatically operated camera supported on a pillar stand. The camera was connected to a J35 automatic exposure unit, both supplied by Vickers Ltd., Vickers Instruments, Haxby Road, York.

E. GROWTH EXPERIMENT

One hundred and twenty day-old male broiler chicks supplied by Ross were received on Friday, 3 November 1978. All chicks were wing-band numbered to follow the body weight of each bird throughout the experiment. They were divided into four groups, each of 26 chicks. Four tiers of electrically-heated battery cages were used (see (b) Animals, accommodation and diet, (1) Chicks).

The temperature was monitored daily, and kept within the range 24-32°C. Its variation was shown to be optimal for the age of the broiler chicks studied during the full experimental period.

The photo period was 24 hours continuously and was held constant throughout the experiment. It has been shown (Moore, 1957; Schutz et al, 1960; Kruger et al, 1963; Beane, et al, 1962, 1965) that broilers grew heavier when exposed to continuous lighting at a single intensity than those exposed to either varying intensities or intermittent light and darkness. Weaver et al (1969) and Deaton and Reece (1975) also reported that male broilers grown under continuous lighting were significantly heavier at 8 weeks of age than other males exposed to a period of darkness.
(a) **Diets**

All chicks were fed commercial diet from 1-7 days old. Its composition is shown in Table 2.1. The diet contained satisfactory levels of crude protein, metabolisable energy and amino acids for broiler chicks, NRC (1971). From 7 days old onwards four different diets were offered. Tables 2.7 and 2.8 give their formulation and chemical composition, including that of the primary diet. Beecham Computing Unit kindly computed the composition of the primary diet.

Amino acid analyses were calculated using the figures produced by the Agricultural Research Council (ARC, 1975).

The main reason for using the computer was to prepare a diet containing half the recommended level of methionine and cysteine for broiler chicks. Then synthetic DL-methionine and L-cysteine were used to make up the desired levels as shown in Table .

The primary diet was supplied in a 700 kg batch (C A Botting and Sons Ltd., Albury Mill, Albury, Surrey). In order to make up 50 kg experimental batches, the formulation of which is shown in Table 2.7, 4 kg of the primary diet was used to mix in the desired quantities of DL-methionine, L-cysteine or both. When sufficiently mixed in a small mixer, the 4 kg of this carrier diet was added to the remaining 46 kg of primary diet and finally mixed in a larger capacity mixer. This gave the most satisfactory distribution and homogenisation of the added amino acids.

From the time the chicks were 35-56 days old they were given the commercial broiler diet which was used during the first seven days of the experiment.


<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control</th>
<th>Diet A</th>
<th>Diet B</th>
<th>Primary diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat meal</td>
<td>71.7</td>
<td>71.7</td>
<td>71.7</td>
<td>71.7</td>
</tr>
<tr>
<td>Barley meal</td>
<td>8.7</td>
<td>8.7</td>
<td>8.7</td>
<td>8.7</td>
</tr>
<tr>
<td>Groundnut extract</td>
<td>7.3</td>
<td>7.3</td>
<td>7.3</td>
<td>7.3</td>
</tr>
<tr>
<td>Maize meal</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Fat 50%</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>L-lysine*</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Salt</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>DL-methionine*</td>
<td>0.2</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-cysteine</td>
<td>0.23</td>
<td>-</td>
<td>0.23</td>
<td>-</td>
</tr>
</tbody>
</table>

* DL-methionine was used because it has the same biological value as L-methionine (Fell et al, 1959). Also the form of lysine used was L-lysine since the D-form antagonizes the action of the L-form and acts as a growth depressant (Fell et al, 1959).
TABLE 2.8 Chemical composition of the primary diet for broiler chicks

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>13.60</td>
</tr>
<tr>
<td>Oil</td>
<td>4.39</td>
</tr>
<tr>
<td>Fibre</td>
<td>3.06</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.50</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.20</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.23</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.35</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.40</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.13</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.10</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>0.98</td>
</tr>
<tr>
<td>Salt</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>ME kcal/kg</strong></td>
<td><strong>3007</strong></td>
</tr>
</tbody>
</table>
(b) Food consumption

Throughout the experiment the chicks were offered food ad libitum. Mean weekly food consumption per chick for eight consecutive periods during the course of investigation were recorded.

(c) Body weight

The individual body weight of all birds in the experiment was measured and recorded periodically through the experiment on days 1, 7, 14, 21, 28, 35, 42, 49 and 56. The weight was recorded to the nearest gram. While the body weights were recorded the food troughs were removed from all cages since the total time of recording weight was one hour.
CHAPTER 3

THE EFFECT OF AGE AND BODY WEIGHT ON AMINO ACID ABSORPTION IN THE RAT AND CHICK
A. INTRODUCTION

The rate of absorption of various amino acids in the chick and rat varies inversely with the molecular volume of the compound (Verzar and McDougall, 1936; Kratzer, 1944). Each amino acid has a characteristic absorption rate (Wiseman, 1956) but this rate varies with the presence of other amino acids, proving the existence of competition within the transport mechanisms (Wiseman, 1955; Agar et al., 1956; Hagihire et al., 1960). Reiser and Christiansen (1971a, 1971b, 1971c) have firmly established that mutual inhibition exists between basic amino acids and those neutral amino acids which at certain concentrations stimulate different factors involved in the transport of basic amino acids. There is, however, a group of neutral amino acids which are rather potent inhibitors of lysine transport, whose own transport is not easily, or may not be at all inhibited by lysine. Munck and Rasmussen (1975) studied in detail this type of interaction for tryptophan and lysine. They indicated that tryptophan is not transported by, but immobilises, the lysine carrier in the brush border of the cell membrane. In addition, lysine and tryptophan appear to have an ordinary competitive relationship with the lysine carrier of the basolateral membrane.

In vitro studies show that most L-amino acids pass through cellular membranes of the intestine against a concentration gradient, a fact which indicates that active transport takes place (Wiseman, 1951; Agar et al., 1953). Some amino acids of the D-form are also transported actively (De La et al., 1971). In the in vivo situation the absorption of free amino acids seems to be slightly quicker than that of the amino acids coming from proteins (Pion et al., 1972;
Rols et al., 1969, 1972) which is in agreement with the findings concerning the kinetics of disappearance of free amino acids from the digestive tract.

The form of stereoisomer administered to a rat may have some effect on the absorption rate. The D-form is generally slower than the L-form (Matthews and Smyth, 1954) except in the case of methionine, which shows no obvious difference between the two forms (Kalafian, 1970).

It seems important to study the absorption characteristics of amino acids at different ages for it is well known that amino acid requirements depend on age. In general it is considered that requirements decrease as the animal matures. Nevertheless this principle cannot be applied to all amino acids. This has been pointed out by many workers in the past. The decrease of absorption with age does not occur with the same intensity for each amino acid. No systematic study of the relationship between age and amino acid absorption could be found in the literature, particularly for the chick. This chapter describes studies that have been done to demonstrate the effect of age on amino acid absorption in the rat and the chick.

B. RESULTS

Under the conditions prevailing in our experiments the intestines from all groups of rats were able to transfer all the amino acids used into the serosal fluid against a concentration gradient. The initial ratio of serosal to mucosal concentration for all amino acids was 1:1. The final concentration of the amino acid in the serosal fluid was well above its initial concentration.
1. Rats

(i) L-Alanine. The absorption rate of L-alanine (Table 3.1) tended to fall after 30 days of age but the difference between the rate at 60 days and that at 21 days was not statistically significant. However, the concentration gradient rose between 21 and 30 days of age and at 30 days the difference was significant (P < 0.02). The value remained at approximately the same level through to 60 days when it was still significantly higher than at 21 days (P < 0.05).

(ii) L-Methionine. In contrast to the results for L-alanine, the absorption of L-methionine (Table 3.2) fell abruptly between 21 and 25 days of age but did not change during the subsequent period of study. The concentration gradient for L-methionine, like that for L-alanine, rose with age, and in the case of methionine it rose sharply between 21 and 25 days (P < 0.05) and continued at approximately the same level throughout the period of study.

(iii) L-Cysteine. There was a higher overall absorption for L-cysteine (Table 3.3) than for L-alanine and L-methionine. The fall in absorption rate of this amino acid which occurred between 21 and 25 days of age was not statistically significant, but the rate continued to fall and by 45 days the difference was highly significant (P < 0.001). The concentration gradient remained at approximately the 21 day level through to 30 days, but by 45 days was significantly lower and did not change further through to 60 days.

(iv) Comparison of amino acids. Fig. 3.1 illustrates the differences between the absorption rates of these three amino acids.
At 21 days of age the absorption of L-cystine was approximately 1.5 times that of methionine and twice that of L-alanine. At 60 days of age the value for L-cysteine was still higher than that for either of the other two amino acids and when compared with L-alanine the difference was still significant (P < 0.05).

Fig. 3.2 shows that the concentration gradient for cysteine was always higher than that for the other two amino acids and that the gradient for alanine was intermediate between that for cysteine and methionine. The concentration gradients for alanine and methionine therefore showed a different pattern from the absorption rate (Fig. 3.2).

2. **Chicks**

(i) **L-alanine.** The rate of transport of L-alanine fell significantly with age between 5 and 35 days of age (Table 3.4). The concentration gradient showed less significant differences when all groups were compared with chicks of 5 days of age. However, the chicks at 11 and 16 days of age had a significantly lower serosal/mucosal ratio than at either 5 or 8 days. This transient fall in the concentration gradient was examined in more detail by carrying out similar measurements at daily intervals between 10 and 20 days of age. The chicks were weighed and carefully allocated to groups of five for killing on successive days. They were matched for body weight in such a way that the group killed on day 10 could be assumed to represent the starting point for those killed subsequently. As before, there was a fall in the rate of absorption with increasing age. There was a tendency for the concentration gradient to fall between 11 and 14 days and the values at 12 and 14 days were significantly lower (P < 0.02).
TABLE 3.1  Comparison of rate of absorption and concentration gradient for L-alanine in rats of different ages. Values are the means for two samples from each of five animals ± S.E. of the mean

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Body weight (g)</th>
<th>Absorption μmole/100 mg dry defatted free tissues</th>
<th>Concentration gradient Ratio Serosal conc. Mucosal conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>37.44 ± 1.28</td>
<td>7.33 ± 0.37</td>
<td>7.74 ± 0.69</td>
</tr>
<tr>
<td>25</td>
<td>45.42 ± 1.01</td>
<td>7.13 ± 0.23&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td>9.00 ± 0.43&lt;sup&gt;n.s.&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>72.22 ± 2.20</td>
<td>7.23 ± 0.36&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td>10.29 ± 0.41**</td>
</tr>
<tr>
<td>45</td>
<td>157.30 ± 2.10</td>
<td>6.64 ± 0.73&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td>9.84 ± 0.61&lt;sup&gt;n.s.&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>258.54 ± 0.90</td>
<td>6.10 ± 0.31&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td>10.05 ± 0.45*</td>
</tr>
</tbody>
</table>

Significance of differences between values at 21 days old and all other groups:

n.s. - not significant;  * - P < 0.05;  ** - P < 0.02
### TABLE 3.2
Comparison of rate of absorption and concentration gradient for L-methionine in rats of different ages. Values are the means for two samples from each of five animals ± S.E. of the mean.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Body weight (g)</th>
<th>Absorption μmole/100 mg dry defatted free tissues</th>
<th>Concentration gradient Ratio</th>
<th>Serosal conc. Mucosal conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>41.34 ± 0.56</td>
<td>10.98 ± 1.16</td>
<td>7.22 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>47.92 ± 0.23</td>
<td>7.68 ± 1.51*</td>
<td>8.54 ± 0.67*</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>75.90 ± 0.86</td>
<td>6.92 ± 0.27*</td>
<td>9.07 ± 0.62*</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>159.20 ± 1.27</td>
<td>7.33 ± 0.19*</td>
<td>8.88 ± 0.11*</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>255.40 ± 0.75</td>
<td>7.15 ± 0.49*</td>
<td>8.70 ± 0.54*</td>
<td></td>
</tr>
</tbody>
</table>

Significance of differences between values at 21 days old and all other groups.

n.s. ; not significant; * - P < 0.05
TABLE 3.3  Comparison of rate of absorption and concentration gradient for L-cysteine in rats of different ages. Values are the means for two samples from each of five animals ± S.E. of the mean.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Body weight (g)</th>
<th>Absorption μmole/100 mg dry defatted free tissues</th>
<th>Concentration gradients Ratio</th>
<th>Serosal conc.</th>
<th>Mucosal conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>42.80 ± 0.20</td>
<td>14.89 ± 0.14</td>
<td>14.19 ± 0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>47.80 ± 0.20</td>
<td>13.67 ± 0.51&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td>13.86 ± 0.39&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>71.80 ± 0.20</td>
<td>11.46 ± 0.33&lt;sup&gt;***&lt;/sup&gt;</td>
<td>13.75 ± 0.40&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>158.60 ± 0.25</td>
<td>8.79 ± 0.45&lt;sup&gt;***&lt;/sup&gt;</td>
<td>11.57 ± 0.83&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>259.00 ± 0.45</td>
<td>7.98 ± 0.25&lt;sup&gt;***&lt;/sup&gt;</td>
<td>11.67 ± 0.34&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significance of differences between values at 21 days old and all other groups:

n.s. - not significant; * - P < 0.05; ** - P < 0.02; *** - P < 0.01; **** - P < 0.001
FIG. 3.1 Comparison of rate of absorption between L-alanine, L-cysteine and L-methionine in rats of different ages. Values are the mean for two samples from each of five animals.
FIG. 3.2 Comparison of concentration gradient between L-alanine, L-cysteine and L-methionine in rats of different ages. Values are the mean for two samples from each of five animals.
### TABLE 3.4 Comparison of rate of absorption and concentration gradient for L-alanine in chicks of different ages. Values are the means for two samples from each of five animals ± S.E. of the mean

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Body weight (g)</th>
<th>Absorption ( \mu \text{mole/100 mg dry defatted free tissues} )</th>
<th>Concentration gradient Ratio</th>
<th>Serosal conc.</th>
<th>Micosal conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>50.02 ± 0.32</td>
<td>7.25 ± 0.35</td>
<td>7.73 ± 0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>67.62 ± 0.84</td>
<td>5.79 ± 0.43(^{n.s.})</td>
<td>8.18 ± 0.38(^{n.s.})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>100.00 ± 2.66</td>
<td>5.14 ± 0.15(^{***})</td>
<td>5.93 ± 0.19(^{**})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>167.40 ± 1.12</td>
<td>5.34 ± 0.34(^{*})</td>
<td>5.48 ± 0.28(^{***})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>210.00 ± 2.95</td>
<td>6.14 ± 0.49(^{n.s.})</td>
<td>8.19 ± 0.60(^{n.s.})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>263.40 ± 2.60</td>
<td>5.50 ± 0.30(^{n.s.})</td>
<td>7.59 ± 0.43(^{n.s.})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>300.44 ± 1.35</td>
<td>5.30 ± 0.48(^{***})</td>
<td>9.53 ± 0.94(^{n.s.})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>512.10 ± 3.62</td>
<td>4.12 ± 0.36(^{****})</td>
<td>6.57 ± 0.31(^{n.s.})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>670.30 ± 3.72</td>
<td>4.78 ± 0.27(^{****})</td>
<td>8.61 ± 0.27(^{n.s.})</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significance of differences between values at 5 days old and all other groups:

n.s. - not significant;  * - \( P < 0.05 \);  ** - \( P < 0.02 \);  *** - \( P < 0.01 \);  **** - \( P < 0.001 \)
TABLE 3.5 Comparison of rate of absorption and concentration gradient for L-alanine in chicks from 10-20 days of age. Values are the means for two samples for each of five animals ± S.E. of the mean

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Body weight (g)</th>
<th>Absorption μmole/100 mg dry defatted free tissues</th>
<th>Concentration gradients Ratio Serosal conc. Mucosal conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>81.0 ± 0.82</td>
<td>6.66 ± 0.16</td>
<td>8.46 ± 0.40</td>
</tr>
<tr>
<td>11</td>
<td>89.2 ± 0.70</td>
<td>4.88 ± 1.27*</td>
<td>6.69 ± 0.71n.s.</td>
</tr>
<tr>
<td>12</td>
<td>94.8 ± 2.18</td>
<td>4.70 ± 0.40**</td>
<td>7.23 ± 0.51**</td>
</tr>
<tr>
<td>13</td>
<td>115.6 ± 1.21</td>
<td>6.46 ± 0.73n.s.</td>
<td>8.49 ± 0.95n.s.</td>
</tr>
<tr>
<td>14</td>
<td>122.6 ± 1.94</td>
<td>4.29 ± 0.20****</td>
<td>6.78 ± 0.43***</td>
</tr>
<tr>
<td>15</td>
<td>132.2 ± 1.59</td>
<td>4.39 ± 0.42****</td>
<td>8.50 ± 1.02n.s.</td>
</tr>
<tr>
<td>16</td>
<td>151.8 ± 1.36</td>
<td>5.64 ± 0.29n.s.</td>
<td>10.98 ± 0.34***</td>
</tr>
<tr>
<td>17</td>
<td>172.2 ± 2.27</td>
<td>4.47 ± 0.17****</td>
<td>8.93 ± 0.94n.s.</td>
</tr>
<tr>
<td>18</td>
<td>173.0 ± 2.68</td>
<td>5.79 ± 1.00n.s.</td>
<td>11.48 ± 0.52***</td>
</tr>
<tr>
<td>19</td>
<td>194.8 ± 1.99</td>
<td>5.32 ± 0.29**</td>
<td>11.99 ± 0.66***</td>
</tr>
<tr>
<td>20</td>
<td>226.2 ± 5.37</td>
<td>4.23 ± 0.33***</td>
<td>10.10 ± 0.86n.s.</td>
</tr>
</tbody>
</table>

Significance of differences between values at 10 days of age and all other groups:

n.s. - not significant; * - P < 0.05; ** - P < 0.02; *** - P < 0.01; **** - P < 0.001
and < 0.01 respectively) than at 10 days. At 15 days the value was about the same as at 10 days with a tendency for values at subsequent ages to be significantly higher than at 10 days.

(ii) **L-Methionine.** The rate of absorption of L-methionine (Table 3.6) followed the same pattern as that of L-alanine, that is, the values fell steadily with increasing age. The mean values at each age were, however, somewhat lower than those for L-alanine. The concentration gradient did not change between 5 and 8 days. The mean value at 11 days was higher but the difference was not significant. By 16 days the value had risen still further and the difference between the concentration at 16 days (10.87 ± 0.27) and that at 5 days (7.33 ± 0.51) was highly significant (P < 0.01). The concentration gradient remained at about this value through to 35 days.

(iii) **L-Cysteine.** The intestinal absorption and concentration gradient for L-cysteine were considerably higher than those of the other amino acids in this study (Table 3.7). The values for chicks of 5 days of age were about four times higher than those of L-alanine or L-methionine. The mean rate of absorption fell steadily from 5 days of age but the differences between the values did not attain statistical significance until 19 days of age. The concentration gradient did not change significantly between 5 and 8 days but the value at 11 days was significantly higher. The values tended to remain at the same level through to 35 days.

(iv) **L-Lysine.** This amino acid had the lowest value for the rate of absorption when compared with the values for the other amino
acids (Table 3.8). The absorption rate did not change between 5 and 22 days but had fallen by 25 days and tended to continue to fall through to 35 days. The concentration gradient did not change significantly between 5 and 16 days but by 19 days the value had risen significantly and it remained at about the same value through to 35 days of age.

(v) L-Lysine + L-Methionine. The results for the absorption of an equimolar mixture of these two amino acids are shown in Table 3.9. The absorption rate showed little change with increasing age with values higher than those at 5 days being found only at 11, 16 and 25 days. The values for methionine and lysine alone are shown in Table 3.10 compared with those for the mixture. They are also shown in Fig. 3.3. It can be seen that the pattern of absorption with age of the mixture was different from that of either amino acid alone because the absorption of both amino acids when present singly fell significantly with increasing age. Furthermore, the values for the mixture were not always intermediate between those obtained for the single amino acids and at most ages more nearly approximated to those of L-lysine.

The concentration gradient (Table 3.9 and Fig. 3.4) did not change between 5 and 8 days of age but by 11 days had risen significantly. The gradient continued to rise to 19 days and then remained at about this level through to 35 days. The concentration gradient for the mixture of amino acids was lower than those of either of the components individually until 11 days of age. After this age the values were similar to those of each amino acid when present alone.

(yi) Comparison of amino acids. When the absorption rates for the three amino acids were compared (Fig. 3.1) it was found that in
the rat the absorption rate for cysteine was always considerably higher than that for either of the other two amino acids. In the chick the absorption rates for alanine and methionine were almost indistinguishable (Fig. 3.5). Unlike the rat, the concentration gradient for methionine (Fig. 3.6) in the chick, from 12 days onwards, was always higher than that for alanine.

3. Comparison of rats and chicks

(i) L-Alanine. The rate of absorption of L-alanine in the chick at 5 days of age was similar to that in the rat at 21 days (Fig. 3.7). However, the rate in the chick then decreased significantly with age whereas that in the rat showed a rather smaller decrease. The decrease in the chick appeared not to be a gradual process for there was a period between 11 and 25 days when no fall occurred. The concentration gradient in rats and chicks similarly started at about the same value (Fig. 3.8). In the rat, the gradient rose to 30 days of age and did not change subsequently. This apparently regular curve was in contrast to that found for the chick for in this species the gradient fell between 8 and 16 days and then rose again.

(ii) L-Methionine. The absorption rate in the chick at 5 days for methionine (Fig. 3.9) was lower than that in the rat at 21 days, and this difference continued in the curves for both species fell with increasing age. In contrast, the concentration gradient for methionine in the chick (Fig. 3.10), though initially at a similar level to that in the rat, rose sharply after 8 days and from 11 days was always higher than in the rat. In both species the gradient
appeared to reach a plateau. In the chicks this was from 19 days onwards and in the rat from 30 days.

(iii) L-Cysteine. The absorption rate for L-cysteine, though falling with age in the chick (Fig. 3.11), was always higher than in the rat. The concentration gradient for this amino acid (Fig. 3.12) was also always higher in the chick than in the rat. Moreover, the gradient increased with age in the chick up to 16 days, whereas there was a tendency for the gradient to fall with age in the rat.

C. DISCUSSION

The results of all the experiments in both chicks and rats showed that when individual amino acids are introduced into an intestine gut sac separately, each amino acid is absorbed at a characteristic rate. The capacity for absorption of all amino acids with the exception of lysine in the chick declined with age in both species. These results are in agreement with those of Wiseman (1955) for the rat. No previous studies of this kind could be found for chicks.

The absorptive cell does not represent a single absorptive barrier. Active transport of amino acids across the epithelium can be divided into transport across the luminal membrane from the intestinal lumen into the cell where the amino acid may be concentrated up to ten times or more. There is then transport across the cell and movement out of the cell on the serosal side, which does not require energy (Smyth, 1967). The carrier hypothesis is perhaps the most popular model to explain the phenomenon of active transport across the cell membrane. However, a number of alternative theories have been
proposed by Lings (1962), including the suggestion that active accumulation by the columnar cells is primarily a property of the cytoplasm rather than the cell membrane.

It was advantageous to keep the volume of fluid on the mucosal side as large as possible in relation to the serosal side, so as to reduce to a minimum any changes in mucosal amino acid concentration during the experimental period. The small volume of fluid contained in the sac allowed a rapid rise in the concentration of transferred amino acid. However, some of the initial work showed that too violent shaking of the sac, i.e. 90 oscillations per minute or more, during incubation can damage the mucosal surface and thus lead to inaccurate absorption results due to intestinal villi clumping together, or even peeling off the mucosal surface. It is surprising that shaking the samples at 90 oscillations per minute, first described by Wiseman and Wilson (1954), is still used in more recent experiments (Hindmarsh et al, 1967). In this experiment all samples were shaken at 40 oscillations per minute. This was thought to be ample for the sample movements during incubation, representing the physiological movement of the intestine in the live animal. Fewer oscillations per minute ensures no intestinal tissue damage.

In rats the rate of absorption of L-methionine was higher than that of L-alanine, while in chicks this was reversed. This difference may explain the higher dietary methionine requirement in chicks. Also the rate of absorption of L-methionine in rats was almost twice as much in the early stage compared with chicks. It seems that L-methionine is absorbed faster and more freely in mammals than in the chick. It must, however, be borne in mind that absorption of
methionine is only one aspect of methionine metabolism. It is possible that methionine turnover is faster in mammals compared with that in the avian. It was also noted that the rate of absorption of L-methionine is lower than L-alanine in the chicks. This is in agreement with Lin et al (1962). They showed a lower absorption for L-methionine when compared with L-alanine. However, the rate of absorption of cysteine in chicks was higher than that in the rat. This shows that even the sulphur amino acids, methionine and cysteine, have their own characteristic absorption. While interpreting these results it is important to bear in mind that methionine can be converted to cysteine within the cell, although not vice-versa.

It is also suggested that free cysteine may be more efficiently absorbed in the jejunum of both the chick and rat than methionine. The study of free individual amino acid absorption here is very useful compared with that of amino acids, especially for sulphur amino acids.

L-lysine showed that lowest absorption rate and concentration gradient. It was suggested by Wiseman (1954) that L-lysine is not actively transported. If this were so it would be expected that there would be no concentration gradient from the mucosal to the serosal surface. The transport of L-lysine in the rat was not studied in the present experiment, but in the chick it was found that the gradient rose from 7.40 to 11.50 between 5 and 35 days of age (Table 3.8). It therefore appears that active transport of L-lysine does occur in the growing chick.
It must be borne in mind that when free amino acids are present, the absorption kinetics are different from those of the amino acids from the natural diet. The rate of absorption of free amino acids is more rapid than those derived from protein hydrolysation (Pion et al, 1972; Rolls et al, 1972).

It was also shown (Table 3.9) that the mixture of amino acid showed some kind of interference and competition. This is in agreement with Pinsky and Geiger (1952) and Wiseman (1955) that when individual amino acids were introduced into the intestinal lumen separately, each amino acid was absorbed at a characteristic rate. However, these absorption rates vary with the presence of other amino acids, and this fact indicates that amino acids compete with each other for their absorption. There is a different argument also that could come into this discussion, for Wilson (1962) stated that all basic amino acids are transported in the intestine by a mechanism different from that of the monobasic-monocarboxylic amino acids (e.g. methionine). However, Wilson (1962) also stated that L-cysteine is transported by the same mechanism as that of basic amino acids. It has been shown also by Agar et al (1954), Finch and Hird (1960), and Nathans et al (1960) that dibasic amino acids have no effect on the intestinal absorption of neutral amino acids. However, the effect of neutral amino acids on the absorption of dibasic amino acids has not been studied in detail.

In these studies opportunity has been taken to compare the change in the absorption characteristics of three amino acids in a mammal (the rat) and in an avian (the chick). It was of interest, therefore, to compare the changes with age in these characteristics
with respect to the three amino acids, alanine, methionine and cysteine. For L-alanine the absorption rate and concentration gradient generally followed a similar pattern in each species, with both curves for the chick being below those for the rat. However, this was not true of the sulphur amino acids for the concentration gradients of both these amino acids rose in the chick. This showed that the intestine of the chick had an increasingly greater ability with age to absorb the sulphur amino acids against a concentration gradient. It is tempting to suggest that these species' differences in the absorption properties of the intestine reflect the different requirements of the rat and chick for sulphur amino acids.

It was reported by Rose et al (1948) that cysteine was classified as a non-essential amino acid with respect to its growth effect in the rat. Also they reported that cysteine can replace about one-sixth of the methionine requirement, but has no growth effect in the absence of methionine. However, in chicks cysteine could supply approximately two-thirds of the total sulphur amino acid requirement, but 0.28% of methionine in the diet was irreplaceable. (West et al, 1951). It is also reported by DSM (1965) that cysteine is classified as an essential amino acid and could replace 50% of the methionine requirement. These findings are in agreement with the results obtained from this experiment which showed a high rise in absorption rate and concentration gradient in the chick experiments when compared with rats.
TABLE 3.6 Comparison of rate of absorption and concentration gradient for L-methionine in chicks of different ages. Values are the means for two samples from each of five animals ± S.E. of the mean

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Body weight (g)</th>
<th>Absorption μmole/100 mg dry defatted free tissues</th>
<th>Concentration gradients Ratio Serosal conc. Mucosal conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>47.60 ± 0.25</td>
<td>6.78 ± 0.24</td>
<td>7.33 ± 0.51</td>
</tr>
<tr>
<td>8</td>
<td>60.20 ± 0.74</td>
<td>5.23 ± 0.49*</td>
<td>6.74 ± 0.55n.s.</td>
</tr>
<tr>
<td>11</td>
<td>108.80 ± 0.22</td>
<td>4.25 ± 0.42**</td>
<td>8.95 ± 0.16n.s.</td>
</tr>
<tr>
<td>16</td>
<td>170.60 ± 2.04</td>
<td>5.63 ± 0.51n.s.</td>
<td>10.87 ± 0.27***</td>
</tr>
<tr>
<td>19</td>
<td>244.60 ± 1.94</td>
<td>5.50 ± 0.25n.s.</td>
<td>13.98 ± 0.46****</td>
</tr>
<tr>
<td>22</td>
<td>265.40 ± 2.04</td>
<td>4.78 ± 0.51**</td>
<td>11.28 ± 0.48***</td>
</tr>
<tr>
<td>25</td>
<td>347.60 ± 3.20</td>
<td>5.36 ± 0.45**</td>
<td>12.94 ± 0.14*</td>
</tr>
<tr>
<td>30</td>
<td>517.50 ± 6.87</td>
<td>3.91 ± 0.37***</td>
<td>13.55 ± 0.98**</td>
</tr>
<tr>
<td>35</td>
<td>630.14 ± 7.00</td>
<td>3.70 ± 0.48***</td>
<td>13.22 ± 1.00**</td>
</tr>
</tbody>
</table>

Significance of differences between values at 21 days old and all other groups:

n.s. - not significant; * - P < 0.05; ** - P < 0.02; *** - P < 0.01; **** - P < 0.001
### TABLE 3.7
Comparison of rate of absorption and concentration gradient for L-cysteine in chicks of different ages. Values are the means for two samples from each of five animals ± S.E. of the mean.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Body weight (g)</th>
<th>Absorption (μmole/100 mg dry defatted free tissues)</th>
<th>Concentration gradient Ratio</th>
<th>Serosal conc.</th>
<th>Micosal conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>48.40 ± 0.25</td>
<td>32.76 ± 2.06</td>
<td>28.71 ± 1.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>65.80 ± 0.20</td>
<td>29.38 ± 0.52&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td>29.38 ± 1.86&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>105.00 ± 0.45</td>
<td>27.97 ± 2.54&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td>35.05 ± 1.02&lt;sup&gt;****&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>152.60 ± 1.12</td>
<td>25.70 ± 2.02&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td>41.09 ± 2.60&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>215.80 ± 1.96</td>
<td>18.95 ± 0.87&lt;sup&gt;***&lt;/sup&gt;</td>
<td>35.31 ± 0.72&lt;sup&gt;***&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>255.80 ± 1.80</td>
<td>16.46 ± 0.15&lt;sup&gt;***&lt;/sup&gt;</td>
<td>40.44 ± 1.85&lt;sup&gt;***&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>302.40 ± 2.69</td>
<td>13.57 ± 1.01&lt;sup&gt;***&lt;/sup&gt;</td>
<td>31.99 ± 2.14&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>585.50 ± 3.18</td>
<td>14.79 ± 0.56</td>
<td>40.32 ± 2.49&lt;sup&gt;****&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>640.00 ± 4.15</td>
<td>13.10 ± 0.79&lt;sup&gt;***&lt;/sup&gt;</td>
<td>39.40 ± 2.20&lt;sup&gt;****&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significance of differences between values at 5 days old and all other groups:

- n.s. - not significant;  *** - P < 0.01;  **** - P < 0.001
TABLE 3.8 Comparison of rate of absorption and concentration gradient for L-lysine in chicks of different ages. Values are the means for two samples from each of five animals ± S.E. of the mean

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Body weight (g)</th>
<th>Absorption ( \mu )mole/100 mg dry defatted free tissues</th>
<th>Concentration gradient Ratio Serosal conc. Mucosal conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>41.00 ± 0.00</td>
<td>3.15 ± 0.35^n.s.</td>
<td>7.40 ± 1.04</td>
</tr>
<tr>
<td>8</td>
<td>62.00 ± 0.70</td>
<td>3.10 ± 0.29^n.s.</td>
<td>7.05 ± 0.58^n.s.</td>
</tr>
<tr>
<td>11</td>
<td>94.50 ± 1.02</td>
<td>3.01 ± 0.18^n.s.</td>
<td>7.00 ± 0.46^n.s.</td>
</tr>
<tr>
<td>16</td>
<td>138.90 ± 1.20</td>
<td>2.90 ± 0.16^n.s.</td>
<td>8.43 ± 0.60^n.s.</td>
</tr>
<tr>
<td>19</td>
<td>208.00 ± 2.00</td>
<td>3.53 ± 0.32^n.s.</td>
<td>10.92 ± 0.39^*</td>
</tr>
<tr>
<td>22</td>
<td>249.00 ± 3.20</td>
<td>3.36 ± 0.29^n.s.</td>
<td>11.26 ± 0.43^**</td>
</tr>
<tr>
<td>25</td>
<td>340.24 ± 2.70</td>
<td>2.44 ± 0.24^*</td>
<td>9.61 ± 0.56^n.s.</td>
</tr>
<tr>
<td>30</td>
<td>550.00 ± 4.00</td>
<td>2.10 ± 0.37^**</td>
<td>10.00 ± 0.38^*</td>
</tr>
<tr>
<td>35</td>
<td>640.00 ± 4.60</td>
<td>2.00 ± 0.30^**</td>
<td>11.50 ± 0.20^**</td>
</tr>
</tbody>
</table>

Significance of differences between values at 5 days old and all other groups:

n.s. - not significant; * - \( P < 0.05 \); ** - \( P < 0.02 \)
### TABLE 3.9
Comparison of rate of absorption and concentration for L-methionine + L-lysine in chicks of different ages. Values are the means for two samples from each of five animals ± S.E. of the mean.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Body weight (g)</th>
<th>Absorption (μmole/100 mg dry defatted free tissues)</th>
<th>Concentration gradients Ratio Serosal conc. Mucosal conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>50.10 ± 0.20</td>
<td>2.50 ± 0.45</td>
<td>3.60 ± 0.47</td>
</tr>
<tr>
<td>8</td>
<td>67.00 ± 0.25</td>
<td>2.18 ± 0.36&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td>3.65 ± 0.52&lt;sup&gt;n.s.&lt;/sup&gt;</td>
</tr>
<tr>
<td>11</td>
<td>98.20 ± 1.40</td>
<td>3.06 ± 0.42**</td>
<td>6.19 ± 0.43***</td>
</tr>
<tr>
<td>16</td>
<td>155.00 ± 2.30</td>
<td>3.75 ± 0.49*</td>
<td>9.34 ± 0.34***</td>
</tr>
<tr>
<td>19</td>
<td>217.10 ± 2.90</td>
<td>3.47 ± 0.55&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td>11.16 ± 0.42****</td>
</tr>
<tr>
<td>22</td>
<td>252.41 ± 3.40</td>
<td>3.05 ± 0.52&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td>11.46 ± 0.28****</td>
</tr>
<tr>
<td>25</td>
<td>320.31 ± 4.10</td>
<td>3.78 ± 0.60*</td>
<td>12.16 ± 0.38****</td>
</tr>
<tr>
<td>30</td>
<td>500.00 ± 5.20</td>
<td>2.20 ± 0.37&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td>12.02 ± 0.59****</td>
</tr>
<tr>
<td>35</td>
<td>620.00 ± 6.30</td>
<td>2.10 ± 0.40&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td>12.10 ± 0.67****</td>
</tr>
</tbody>
</table>

Significance of differences between values at 5 days old and all other groups:
- n.s. - not significant; * - P < 0.05; ** - P < 0.02; *** - P < 0.01; **** - P < 0.001
TABLE 3.10 Comparison of rate of absorption and concentration gradient for L-methionine, L-methionine + L-lysine and L-lysine in chicks of different ages. Values are the means for two samples from each of five animals ± S.E. of the mean.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>L-methionine</th>
<th>L-methionine + L-lysine</th>
<th>L-lysine</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6.78 ± 0.24</td>
<td>2.50 ± 0.45</td>
<td>3.15 ± 0.35</td>
</tr>
<tr>
<td>8</td>
<td>5.23 ± 0.49*</td>
<td>2.18 ± 0.36&lt;sup&gt;n.s&lt;/sup&gt;</td>
<td>3.10 ± 0.29&lt;sup&gt;n.s&lt;/sup&gt;</td>
</tr>
<tr>
<td>11</td>
<td>4.25 ± 0.42**</td>
<td>3.06 ± 0.42**</td>
<td>3.01 ± 0.18&lt;sup&gt;n.s&lt;/sup&gt;</td>
</tr>
<tr>
<td>16</td>
<td>5.63 ± 0.51&lt;sup&gt;n.s&lt;/sup&gt;</td>
<td>3.75 ± 0.49*</td>
<td>2.90 ± 0.16&lt;sup&gt;n.s&lt;/sup&gt;</td>
</tr>
<tr>
<td>19</td>
<td>5.50 ± 0.25&lt;sup&gt;n.s&lt;/sup&gt;</td>
<td>3.47 ± 0.55&lt;sup&gt;n.s&lt;/sup&gt;</td>
<td>3.53 ± 0.32&lt;sup&gt;n.s&lt;/sup&gt;</td>
</tr>
<tr>
<td>22</td>
<td>4.78 ± (0.51)∗∗</td>
<td>3.05 ± 0.52&lt;sup&gt;n.s&lt;/sup&gt;</td>
<td>3.36 ± 0.29&lt;sup&gt;n.s&lt;/sup&gt;</td>
</tr>
<tr>
<td>25</td>
<td>5.36 ± 0.45**</td>
<td>3.78 ± 0.60*</td>
<td>2.44 ± 0.24*</td>
</tr>
<tr>
<td>30</td>
<td>3.91 ± 0.37***</td>
<td>2.20 ± 0.37&lt;sup&gt;n.s&lt;/sup&gt;</td>
<td>2.10 ± 0.30**</td>
</tr>
<tr>
<td>35</td>
<td>3.70 ± 0.48***</td>
<td>2.10 ± 0.40&lt;sup&gt;n.s&lt;/sup&gt;</td>
<td>2.00 ± 0.30**</td>
</tr>
</tbody>
</table>

(Cont)
<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Concentration gradients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ratio serosal conc.</td>
</tr>
<tr>
<td></td>
<td>mucosai conc.</td>
</tr>
<tr>
<td>L-methionine</td>
<td>L-methionine + L-lysine</td>
</tr>
<tr>
<td>L-lysine</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.33 ± 0.51</td>
</tr>
<tr>
<td>8</td>
<td>6.74 ± 0.55&lt;sup&gt;n.s&lt;/sup&gt;</td>
</tr>
<tr>
<td>11</td>
<td>8.95 ± 0.16&lt;sup&gt;n.s&lt;/sup&gt;</td>
</tr>
<tr>
<td>16</td>
<td>10.87 ± 0.27&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>19</td>
<td>13.98 ± 0.46&lt;sup&gt;****&lt;/sup&gt;</td>
</tr>
<tr>
<td>22</td>
<td>11.28 ± 0.48&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>25</td>
<td>12.94 ± 0.14&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>13.55 ± 0.98&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>35</td>
<td>13.22 ± 1.00&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Significance of differences between values at 5 days of age and all other groups:

n.s - not significant; * - P < 0.05; ** - P < 0.02; *** - P < 0.01; **** - P < 0.001
FIG. 3.3 Comparison of rate of absorption for L-lysine, L-methionine and L-lysine + L-methionine in chicks of different ages. Values are the means for two samples from each of five animals.

Chicks' age (days)

mole amino acids absorbed/100 mg defatted dry tissues

- Methionine
- Lysine
- Lysine + Methionine
FIG. 3.4 Comparison of concentration gradient for L-lysine, L-methionine and L-lysine + L-methionine in chicks of different ages. Values are the means for two samples from each of five animals.

- ▼ ▼ Methionine
- ▼ ▼ Lysine
- ■ ■ Lysine + Methionine
FIG. 3.5  Comparison of rate of absorption between L-alanine, L-cysteine and L-methionine in chicks of different ages. Values are the mean for two samples from each of five animals.
FIG. 3.6 Comparison of concentration gradient between L-alanine, L-cysteine and L-methionine in chicks of different ages. Values are the mean for two samples of five animals.
FIG. 3.7 Comparison of rate of absorption for L-alanine in rats and chicks of different ages. Values are the mean for two samples from each of five animals.
FIG. 3.8 Comparison of concentration gradient for L-alanine in rats and chicks of different ages. Values are the mean for two samples from each of five animals.
FIG. 3.9 Comparison of rate of absorption for L-methionine in rats and chicks of different ages. Values are the mean for two samples from each of five animals.
FIG. 3.10 Comparison of concentration gradient for L-methionine in rats and chicks of different ages. Values are the mean for two samples from each of five animals.
FIG. 3.11 Comparison of rate of absorption for L-cysteine in rats and chicks of different ages. Values are the mean for two samples from each of five animals.
FIG. 3.12 Comparison of concentration gradient for L-cysteine in rats and chicks of different ages. Values are the mean for two samples from each of five animals.
CHAPTER 4

THE INTERRELATIONSHIP BETWEEN L-METHIONINE ABSORPTION
AND OTHER FACTORS IN THE BROILER CHICK INTESTINE
A. **INTRODUCTION**

The studies of Wilson (1962) and Wiseman (1968) should be consulted on carrier specificities of intestinal absorption of amino acids. The role of sodium and the energetics of amino acid transport have been discussed in detail (Christensen, 1972; Schultz and Curran, 1970; Kimmich, 1973). Heinz (1972) and LeFevre (1972) studied the transport of sugars and amino acids by mammalian cells in general.

Absorption of sugars takes place readily only from the small intestine. Limited absorption from the stomach has been reported to occur under some conditions (Morrison et al., 1939; Reynell and Spray, 1956) but it is quantitatively unimportant compared to absorption from the small intestine. Using an *in vitro* technique with which active absorption by the small intestine is readily demonstrated, Crane and Mandelstam (1960) found no evidence that hamster stomach or colon are capable of absorbing sugars against a concentration difference.

The absorption process is not uniformly distributed along the length of the intestine. A number of studies have shown that the rate of absorption from the lower ileum is less than from the duodenal and jejunal regions in experimental animals (Magee, 1930; Magee and Reid, 1931).

The active transport of many substances, including amino acids and some monosaccharides, is sodium-dependent (Crane, 1965). According to one hypothesis, sodium and one of the other substrates are linked by a common carrier which equilibrates them across the plasma membrane of the absorptive cell. As sodium enters the carrier, so does the sugar or amino acid, the driving force for movement of the carrier being provided by the electrochemical gradient between
the high extracellular sodium concentration and the low concentration of sodium within the cell. This gradient is maintained by the sodium pump. According to a modification of this hypothesis, the different concentrations of sodium on the two sides of the membrane alter the affinity of the carrier for the substrate in such a way as to produce similar effects. Thus, the active transport of monosaccharides and amino acids into the cell is not directly dependent on metabolic energy, but linked to it through the effects of the sodium pump. The substances accumulated by this means at the mucosal pole of the cell are supposed to diffuse through the cell in the direction of the concentration gradient thus establishing leaving by carriers at the basal pole and the diffusion into the capillaries. As long as the intracellular sodium is kept lower than the extracellular, work can be extracted by this kind of mechanism as sodium passes down the electrochemical gradient into the cell. This arrangement has been referred to as an ionic battery (Parsons, 1967a, 1967b).

As sugars and amino acids share the same sodium dependent absorptive mechanism, it is suggested that a form of inhibition will be developed from one to the other. Newey and Smyth (1964) showed that the actively transferred sugar, galactose, inhibited the intestinal transport of glycine and methionine, and suggested that the cause of inhibition might be competition for a common energy supply or other common requirements for transport. Saunders and Isselbacher (1965) repeated these experiments using intestinal slices of sacs of everted intestine and confirmed the inhibitory effect of galactose on amino acid transfer.

The rate of absorption of amino acids from the gastrointestinal tract of a chick varies inversely with the apparent molar volume of
the amino acid (Kratzer, 1943). However, it can be stated that different concentrations of amino acids in an in vitro system will give different rates of absorption.

The probable ways in which an antibiotic may affect the intestinal flora and promote growth have been listed by Moore et al (1946). Antibiotics may suppress toxin-producers, increase the number of 'synthesizers', and decrease the number of organisms competing for available nutrients. Another possibility would be by decreasing the total weight of the intestinal tract, and decreasing the thickness of the intestinal wall (Coates et al, 1955). In this case absorption might be enhanced because of a thinner wall. The elusiveness of the antibiotic growth response in many laboratories and the complexity of the factors involved are largely responsible for the absence of a satisfactory explanation as to how antibiotics act. For this reason, it is difficult to determine whether the variations in antibiotic growth response are due to changes in the birds or to the environment. The greatest relative growth-stimulation of chicks due to antibiotics was found by Heuser and Norris (1952) during the first four weeks. The differences in weight disappeared as the chicks grew older. A greater relative growth response with antibiotics was obtained in chicks fed vegetable protein rations than in those fed rations containing animal protein. In general, the best weight was obtained with rations containing animal protein supplemented with antibiotics.
B. RESULTS

(1) Effect of time-intervals. The rate of absorption and concentration gradient (Table 4.1) showed very little difference between half and one hour incubation. However, after a two hour incubation period there was a large increase in both the rate of absorption and in the concentration gradient.

(2) Effect of glucose on absorption of L-methionine. Table 4.2 shows the effect of glucose on the rate of absorption and concentration gradient of L-methionine at different ages. The pattern of change with age in these measurements was similar when glucose was included in the medium and when it was not. Methionine showed a higher rate of absorption throughout all the age groups when there was no glucose in the perfusion medium. When the absorption without glucose was compared with that with glucose (Table 4.2), there were significant differences at all ages apart from 22 and 25 days. The same pattern was followed in both absorption rate and concentration gradient although the differences were statistically more significant for the former. The results presented in Table 4.2 are also shown in Figs. 4.1 and 4.2. The effect on the absorption of L-methionine of including a disaccharide, maltose, in the medium was also studied. Table 4.3 shows that although the values for the rate of absorption and concentration gradient for 16-day old chick intestine was lower with maltose, the differences were not statistically significant.

(3) Mixtures of amino acids. Table 4.4 shows comparison of the rate of absorption and concentration gradient of three types of amino acids, acidic, neutral and basic, alone and in mixtures. When present
alone, the absorption rate and concentration gradient for these amino acids was similar. Aspartic acid showed a significantly higher rate of absorption when present alone, compared with when lysine was present with it. Lysine tended also to reduce the concentration gradient. In contrast, methionine had no significant effect on either the absorption or concentration gradient when mixed with aspartic acid. Furthermore, there was significant interaction between lysine and methionine in both absorption and concentration gradients.

(4) **Antibiotics.** It was not possible to do all these experiments on chicks of the same age and for this reason control studies were done at the youngest and oldest ages used (Table 4.5). The values at these ages were not significantly different. Thus, it seems to be legitimate to compare the measurements in the antibiotic treated animals with the nearest age related control. Treatment with each of the drugs significantly reduced the rate of absorption of L-methionine.

In the case of the concentration gradient the differences were less significant, although they still tended to be lower in the treated animals compared with the controls.

(5) **Histological appearances.** Transverse sections of rat and chick intestine were examined histologically. In each of the species there was an increase with age in the diameter of the intestine (Fig. 4.3). At each age the intestine of the rat was smaller than that of the chick. In the rat it appears that there was very little increase with age in the thickness of the muscularis mucosa, whereas there does seem to be some increase between 25 and 30 days of age in the chick. At each age the lamina propria is thinner in the chick.
intestine and the villi correspondingly longer.

There was evidence in all sections of fresh and incubated intestine of slight disengagement of cells from the mucosa at the lips of the villi (Fig. 4.4). There was very little distinction in this respect between fresh and the incubated intestine. This is in agreement with Leblond and Stevens (1948).

C. DISCUSSION

Effect of period of incubation. The intestinal sacs incubated for two hours showed a significantly higher rate of absorption than those at half- and one hour. Since the translocation of amino acids is an active energy-dependent procession, this indicates that the tissue was kept alive up to two hours. The values at half- and one hour were similar. The increase after one hour could have been due to the tissue adapting to its new environment.

In the experiments described in this thesis all incubations were stopped precisely at one hour. It was decided that this would be preferable for practical reasons because it took one hour to prepare 10 sacs and thus as the last sac is put in the bath the first one can be removed. Furthermore, it seemed that there would be less risk of differences between the ages and treatments being due to small differences in the period of incubation if all measurements were made on a steady part of the curve.

Effect of glucose on absorption of L-methionine. The presence of glucose in the incubation medium reduced the rate of absorption of
L-methionine. The most likely reason for this reduction is that glucose was competing with L-methionine for a common carrier. It would seem that using one hour as the incubation time reduced the dependence of the preparation on the availability of exogenous glucose. Thus, glucose did not stimulate amino acid transfer during this period. However, if the incubation had been prolonged to, say, two hours, there could have been a dependence on exogenous glucose and the result might have been different with glucose increasing the absorption rate. These results are in agreement with those of Dawson et al (1965) and Bingham et al (1966) who demonstrated that glucose did not stimulate transfer of methionine. The results of the in vitro experiments reported by Taylor et al (1968) and Reiser and Christiansen (1969) showed that there was increased jejunal inhibition of amino acid transport by galactose, a sugar which is actively transported but, unlike glucose, is not metabolised.

The length of the intestinal sample could play an important role in the glucose inhibition of amino acid transfer. In large sacs (17 cm) glucose activates amino acid transport (Newey and Smyth, 1964; Bingham et al, 1966). In small sacs (7 cm) (Reiser and Christiansen, 1969) glucose did not stimulate amino acid transport. In this work the sac length was fixed at 6 cm for all experiments.

**Antibiotics**

Antibiotics have been said to be animal growth promotors (Hauser and Norris, 1952; Braude et al, 1953; Stokstad et al, 1953). In this work (Table 4.5) the rate of absorption and concentration gradient in all antibiotic-treated groups were lower than those of the controls.
This was a somewhat unexpected result and does not provide any basis for the use of these components as growth promoters, at least insofar as an effect on the absorption of amino acids for tissue growth is concerned. The depression of absorption could be due to smaller villous absorption, similar to that in germ-free animals (Coates et al., 1955). These results suggest that any growth-promoting effect of antibiotics in chicks is probably mediated by a reduction in infection in the chicks. However, it is realised that the period of antibiotic administration in our study was only 3 days whereas longer periods are used in practice.

**Histological differences.** The rate of absorption of some amino acids by the chick intestine was greater than that of the rat intestine at similar ages. This may be explained by the thinner lumina propria and longer villi in the chick intestine. However, the fact that the absorption of all amino acids was not greater in the chick suggests that other factors must also operate such as the requirement of the animal for the particular amino acid. The most notable difference between the species was in the absorption of the sulphur amino acid, cysteine, for which the chick is known to have a high requirement.

In order to establish the effect of incubation on the intestine, a histological comparison of incubated and fresh tissues was made. Very little disengagement of cells from the mucosa was seen in the preparations. This indicated that most of the villi were in working condition and able to absorb the substances presented to them.

**Mixtures of amino acids.** When lysine (a basic amino acid) was mixed with methionine (a neutral amino acid) in the incubation medium the rate of absorption of amino acids was significantly reduced and
was, in fact, similar to that of lysine. This result is in agreement with Robinson (1966; 1968) and Chez et al (1971) who stated that basic and neutral amino acids are transported by the same carrier. Thus there was competition in our experiments with lysine evidently having a stronger effect than methionine, since both were present in equimolar concentrations and in the medium. The absorption of lysine is absolutely sodium-dependent (Reiser and Christiansen, 1972), and it may be that it is this that accounts for its stronger effect.

In the case of aspartic acid (an acidic amino acid) lysine produced the same kind of inhibition and this was in contrast to the effect of methionine. The interaction between lysine and aspartic acid suggests that the movement of acidic amino acid from the intestinal lumen to the serosal side is a carrier-mediated process. It thus appears that acidic amino acids may be translocated by a process similar to that for the neutral amino acids in the chick.
TABLE 4.1 Effect of period of incubation on the rate of absorption and concentration gradient for L-methionine in 22-day old chick intestine. Values are the means for two samples from each of five animals ± S.E. of the mean

<table>
<thead>
<tr>
<th>Incubation time (hr)</th>
<th>Body weight (g)</th>
<th>Absorption μmole/100 mg dry defatted free tissues</th>
<th>Concentration gradients Ratio Serosal conc. Mucosal conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>½</td>
<td>256 ± 2.90</td>
<td>3.08 ± 0.38</td>
<td>11.77 ± 0.37</td>
</tr>
<tr>
<td>1</td>
<td>260 ± 3.40</td>
<td>3.55 ± 0.45&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td>13.72 ± 0.48&lt;sup&gt;n.s.&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>260 ± 3.10</td>
<td>6.07 ± 0.29&lt;sup&gt;***&lt;/sup&gt;</td>
<td>20.00 ± 0.52&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Significance of differences between values of ½ hour and 1- and 2-hour incubation periods:

n.s. - not significant; *** - P < 0.01
**TABLE 4.2** Effect of glucose on the absorption rate and concentration gradient for L-methionine in chicks of different ages. Values are the means for two samples from each of five animals ± S.E. of the mean.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Body weight (g)</th>
<th>Absorption (μmole/100 mg dry defatted free tissues)</th>
<th>Concentration gradients Ratio Serosal conc. Mucosal conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No glucose</td>
<td>With glucose</td>
<td>No glucose</td>
<td>With glucose</td>
</tr>
<tr>
<td>5</td>
<td>42.0 ± 0.0</td>
<td>47.6 ± 0.25</td>
<td>10.38 ± 0.20***</td>
</tr>
<tr>
<td>8</td>
<td>63.2 ± 0.49</td>
<td>60.2 ± 0.74</td>
<td>9.30 ± 0.88***</td>
</tr>
<tr>
<td>11</td>
<td>93.6 ± 0.40</td>
<td>108.8 ± 0.22</td>
<td>9.12 ± 0.49***</td>
</tr>
<tr>
<td>16</td>
<td>142.6 ± 1.35</td>
<td>170.6 ± 2.04</td>
<td>8.05 ± 0.37**</td>
</tr>
<tr>
<td>19</td>
<td>207.0 ± 1.00</td>
<td>244.6 ± 1.94</td>
<td>7.67 ± 0.59**</td>
</tr>
<tr>
<td>22</td>
<td>235.2 ± 2.00</td>
<td>265.4 ± 2.04</td>
<td>5.34 ± 0.25n.s.</td>
</tr>
<tr>
<td>25</td>
<td>360.0 ± 2.90</td>
<td>347.6 ± 3.20</td>
<td>4.55 ± 0.24n.s.</td>
</tr>
<tr>
<td>30</td>
<td>558.7 ± 4.00</td>
<td>517.5 ± 6.87</td>
<td>4.60 ± 0.30*</td>
</tr>
</tbody>
</table>

Significance of differences between values at 21 days of age and all other groups:

n.s. - not significant; * - P < 0.05; ** - P < 0.02; *** - P < 0.01; **** - P < 0.001
FIG. 4.1 Comparison of rate of absorption for L-methionine in chicks of various ages in relation to glucose. Values are the mean for two samples from each of five animals.
FIG. 4.2 Comparison of concentration gradient for L-methionine in chicks of different ages in relation to glucose. Values are the mean for two samples from each of five animals.
<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Body weight (g)</th>
<th>Absorption μmole/100 mg dry defatted free tissues</th>
<th>Concentration gradients Ratio</th>
<th>Serosal conc.</th>
<th>Mucosal conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>99 ± 1.90</td>
<td>5.62 ± 0.32</td>
<td>10.19 ± 0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltose</td>
<td>99 ± 2.00</td>
<td>4.70 ± 0.40&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td>9.11 ± 0.43&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significance of differences between values of glucose and maltose groups:

n.s. - not significant
TABLE 4.4 Comparison of rate of absorption and concentration gradient for L-aspartic acid, L-methionine and L-lysine alone and as mixtures in the intestine of 29-days old chicks. Values are the means for two samples from each of five animals ± S.E. of the mean

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Body weight (g)</th>
<th>Absorption μmole/100 mg dry defatted free tissues</th>
<th>Concentration gradients Ratio</th>
<th>Serosal conc. Mucosal conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid (acidic)</td>
<td>343 ± 5.60</td>
<td>3.16 ± 0.43</td>
<td>10.14 ± 0.50</td>
<td></td>
</tr>
<tr>
<td>Methionine (neutral)</td>
<td>345 ± 6.00</td>
<td>4.08 ± 0.39</td>
<td>10.44 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>Lysine (basic)</td>
<td>344 ± 5.50</td>
<td>3.00 ± 0.46</td>
<td>8.78 ± 0.52</td>
<td></td>
</tr>
<tr>
<td>Methionine + Lysine</td>
<td>350 ± 7.20</td>
<td>2.90 ± 0.42</td>
<td>8.44 ± 0.54</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid + Lysine</td>
<td>350 ± 4.90</td>
<td>2.46 ± 0.48</td>
<td>8.83 ± 0.58</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid + Methionine</td>
<td>345 ± 3.75</td>
<td>3.06 ± 0.28</td>
<td>9.02 ± 0.46</td>
<td></td>
</tr>
</tbody>
</table>

Significance of differences

Aspartic acid vs. Aspartic acid + Lysine       *   *
Aspartic acid vs. Aspartic acid + Methionine   n.s.  n.s.
Methionine vs. Methionine + Lysine            *   *
Methionine vs. Methionine + Aspartic acid      *   *
Lysine vs. Lysine + Aspartic acid              n.s.  n.s.
Lysine vs. Methionine + Aspartic acid          n.s.  n.s.

Significance of differences between individual and mixture groups:

n.s. - not significant;  * - P < 0.05
TABLE 4.5  Effect of giving antibiotics to chicks on the rate of absorption and concentration gradient of L-methionine in the everted gut sacs. Comparison of rate of absorption and concentration gradient

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age (days)</th>
<th>Body weight (g)</th>
<th>Absorption μmole/100 mg dry defatted free tissues</th>
<th>Concentration gradients Ratio Serosal conc. Mucosal conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1)</td>
<td>31</td>
<td>460 ± 5.80</td>
<td>3.27 ± 0.40</td>
<td>15.69 ± 0.51</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>31</td>
<td>466 ± 6.10</td>
<td>2.72 ± 0.46*</td>
<td>10.24 ± 0.47*</td>
</tr>
<tr>
<td>Penicillin</td>
<td>32</td>
<td>470 ± 6.00</td>
<td>2.62 ± 0.35*</td>
<td>11.05 ± 0.49*</td>
</tr>
<tr>
<td>Puromycin</td>
<td>32</td>
<td>470 ± 6.50</td>
<td>2.52 ± 0.40**</td>
<td>13.19 ± 0.51&lt;sup&gt;n.s.&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (2)</td>
<td>33</td>
<td>500 ± 4.90</td>
<td>3.66 ± 0.45&lt;sup&gt;n.s.&lt;/sup&gt;</td>
<td>16.80 ± 0.41&lt;sup&gt;n.s.&lt;/sup&gt;</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>33</td>
<td>510 ± 5.30</td>
<td>2.54 ± 0.67*</td>
<td>14.10 ± 0.39&lt;sup&gt;n.s.&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Significance of differences between values of antibiotics and control groups:

n.s. - not significant;  * - P < 0.05;  ** - P < 0.02
FIG. 4.3 Histological comparison between cross-sections of rat (Al, B1, C1) and chick (A2, B2, C2) intestine at different ages. Magnification x25.
FIG. 4.4. Histological comparison between incubated and non-incubated intestinal tissue of broiler chicks at different ages. Plates A1, B1 and C1 represent sections of incubated tissue and plates A2, B2 and C2 are sections of non-incubated tissue. The magnification of A and B is x100 and that of C is x40.
CHAPTER 5

GROWTH RATE AND SULPHUR AMINO ACID REQUIREMENTS IN CHICKS
A. INTRODUCTION

The experiment described in the previous chapter showed that the absorption rate of cysteine was higher than that of any of the other amino acids studied, moreover the concentration gradient of methionine was also higher than that of other amino acids. It is generally reckoned that the requirement of broiler chicks for sulphur amino acid is high (National Research Council, 1977). Thus, the in vitro measurement made on intestinal gut sac could seem to be in agreement with this high requirement. It was also noteworthy that the result of measurements on chick intestine also suggest this. It was of interest therefore to attempt to demonstrate the dependence of chicks on sulphur amino acids in a growth study in which the concentration of these amino acids in the diet were varied.

Animals and methods

The chicks used and their diets are described in Chapter 2. Four different diets were used, viz:

Diet A - Control diet containing half the requirement for methionine and cysteine from natural ingredients, with the other half being added as free amino acid.

Diet B - The same ingredients as those in Diet A except that there was no addition of free cysteine.

Diet C - The same ingredients as those in Diet A except that there was no addition of free methionine.

Diet D - The same ingredients as those in Diet A except that there was no addition of either methionine or cysteine.
B. RESULTS

During the course of the experiment abnormalities were noticed in some of the chicks which were attributed to Vitamin E deficiency due to the inclusion of too much fat in the diet. As a result of this, the abnormal animals had to be culled and then the size of groups available from 28 days onwards had to be reduced to 12 chicks. Since this was a longitudinal study the body weight of these 12 chicks only were used to calculate the means shown in Table 5.1.

There were no significant differences between treatments throughout all ages. From 35 days of age, at which broiler commercial diet was used, the body weight rose more quickly in relation to the rise at earlier stages. This rise continued up to the end of the experiment at 56 days. Chicks on treatment A showed higher body weight at the end of 35 days but the rise was not significant; this group represented the control diet which contained full requirements for sulphur amino acid. The group on treatment C were second in the order of body weight. At the end of the experiment at 56 days, the animals receiving diet D had the highest mean body weight, even though the difference between it and that of other groups was not statistically significant. The body weights of the animals on all treatments at all ages were variable and thus the mean had a high standard error. The mean daily food consumption per bird for all treatments is shown in Table 5.2 and was similar for all groups at each age.

C. DISCUSSION

It was noticed that body weight (Table 5.1) showed very little difference between the four treatments. This was probably due to the poor primary diet that was used (Table 2.7 and 2.8). The protein
content of the diet was only 13.60% which was low compared with the recommended level by the National Research Council (1977) of 23%. The growth of the chicks was therefore limited by the low protein content of the diet and there was not the expected rate of body weight increase for the added amounts of cysteine and methionine.

Fat at 4.39% was used to put the value of energy in the diet up to 3007 Kcal/kg. It seems that at this level the availability of essential nutrients was not great enough. This was particularly true of Vitamin E which was evidently insufficient for the amount of fat in the diet.

The high energy level of the primary diet may also explain the low feed consumption (Table 5.2) when compared with standard daily food intake for broiler chicks. The ability of chicks to recover from the effects of a poor diet was clearly shown when a broiler commercial diet was offered, for all the chicks recovered to reach a near-expected body weight and food consumption in the final week of the experiment.
TABLE 5.1 Mean body weights during the course of experiments and the S.E. of the mean

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>96 ± 2.84</td>
<td>158 ± 5.76</td>
<td>229 ± 11.41</td>
<td>294 ± 16.22</td>
</tr>
<tr>
<td>B</td>
<td>90 ± 2.45\textsuperscript{n.s}</td>
<td>155 ± 6.41\textsuperscript{n.s}</td>
<td>215 ± 7.74\textsuperscript{n.s}</td>
<td>267 ± 7.48\textsuperscript{n.s}</td>
</tr>
<tr>
<td>C</td>
<td>91 ± 2.37\textsuperscript{n.s}</td>
<td>159 ± 4.96\textsuperscript{n.s}</td>
<td>224 ± 9.76\textsuperscript{n.s}</td>
<td>279 ± 14.04\textsuperscript{n.s}</td>
</tr>
<tr>
<td>D</td>
<td>90 ± 2.08\textsuperscript{n.s}</td>
<td>154 ± 5.10\textsuperscript{n.s}</td>
<td>213 ± 9.17\textsuperscript{n.s}</td>
<td>273 ± 13.18\textsuperscript{n.s}</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 35</th>
<th>Day 42</th>
<th>Day 47</th>
<th>Day 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>359 ± 17.51</td>
<td>536 ± 30.33</td>
<td>882 ± 69.35</td>
<td>1558 ± 71.22</td>
</tr>
<tr>
<td>B</td>
<td>329 ± 10.46\textsuperscript{n.s}</td>
<td>499 ± 15.08\textsuperscript{n.s}</td>
<td>833 ± 21.14\textsuperscript{n.s}</td>
<td>1494 ± 30.18\textsuperscript{n.s}</td>
</tr>
<tr>
<td>C</td>
<td>354 ± 18.36\textsuperscript{n.s}</td>
<td>563 ± 23.92\textsuperscript{n.s}</td>
<td>886 ± 27.72\textsuperscript{n.s}</td>
<td>1563 ± 30.54\textsuperscript{n.s}</td>
</tr>
<tr>
<td>D</td>
<td>331 ± 16.32\textsuperscript{n.s}</td>
<td>560 ± 26.45\textsuperscript{n.s}</td>
<td>906 ± 31.25\textsuperscript{n.s}</td>
<td>1602 ± 41.88\textsuperscript{n.s}</td>
</tr>
</tbody>
</table>

**Diet A** - Control diet contains half the requirement of methionine and cysteine from the natural ingredient; the other half requirement of methionine and cysteine were added as free amino acids.

**Diet B** - The same as those in diet A except that there was no addition of free cysteine.

**Diet C** - The same as those in diet A except that there was no addition of free methionine.

**Diet D** - The same as those in diet A except that there was no addition of either methionine or cysteine.

Significance of differences between values of treatment A and all other groups:

\textsuperscript{n.s} - not significant
TABLE 5.2 Mean daily food consumption (grams) during the course of investigation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
<th>Week 7</th>
<th>Week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12.64</td>
<td>22.77</td>
<td>32.45</td>
<td>34.94</td>
<td>35.83</td>
<td>49.17</td>
<td>69.20</td>
<td>85.61</td>
</tr>
<tr>
<td>B</td>
<td>12.99</td>
<td>23.16</td>
<td>33.48</td>
<td>33.57</td>
<td>34.40</td>
<td>48.49</td>
<td>72.40</td>
<td>85.67</td>
</tr>
<tr>
<td>C</td>
<td>12.61</td>
<td>24.38</td>
<td>30.88</td>
<td>33.80</td>
<td>37.79</td>
<td>50.20</td>
<td>73.21</td>
<td>84.71</td>
</tr>
<tr>
<td>D</td>
<td>12.97</td>
<td>24.91</td>
<td>31.34</td>
<td>34.77</td>
<td>35.33</td>
<td>50.46</td>
<td>70.10</td>
<td>86.28</td>
</tr>
</tbody>
</table>

Diet A - Control diet contains half the requirement of methionine and cysteine from the natural ingredient, the other half requirement of methionine and cysteine were added as synthetic amino acids.

Diet B - The same as those in diet A except that there was no addition of synthetic cysteine.

Diet C - The same as those in diet A except that there was no addition of synthetic methionine.

Diet D - The same as those in diet A except that there was no addition of either synthetic methionine or cysteine.
Methionine has various functions in the animal body. Three of its major roles are as an essential component for protein synthesis, as a methyl donor and as a precursor of cysteine. The pathway for this latter function is also the metabolic pathway for methionine catabolism. A physiological requirement exists for both methionine and cysteine. Hence, when cysteine is deficient and methionine is in excess, methionine is probably converted to cysteine. This conversion achieves two functions: (i) it removes excess methionine which is extremely toxic (Anonymous, 1965), and (ii) it overcomes the deficiency of cysteine.

The reverse of the methionine to cysteine pathway does not occur. Rose and Rice (1939) reported that rats lost weight when fed a methionine-free diet supplemented with cysteine. However, this does not mean that dietary sources of cysteine or cystine cannot be utilized. On the other hand, cysteine has been demonstrated to be capable of replacing part, but not all, of the dietary need for methionine. Considerable research has been conducted to quantitate the maximum amount of the animal's total sulphur amino acid needs that can be supplied by cystine. These estimates have varied depending on species, age, physiological function and criterion of measurement. In general, dietary cystine can contribute approximately 50% of the total sulphur amino acid needs for the young chick and about 80% to 90% for the mature, non-producing bird. It is well recognized that amino acid requirements are positively related to rate of growth, for instance, the poult has a high amino acid requirement per day because it is growing rapidly. The adult male chicken has a substantially lower requirement. When diets are calculated based upon feed composition and analysis information, the
assumption is usually made that amino acids are 80-90 percent available.

It is very important to consider the relationship between amino acid requirement and intestinal absorption. It is known that some of the by-products commonly used in poultry feeds such as feathers or blood are either indigestible in the native form, or made indigestible by over-heating in processing. It seems possible that a combined study of growth rate (in vivo) and intestinal absorption (in vitro) might throw new light on the factors which influence the amino acid requirements of broiler chicks. In this thesis we have examined the effects of growth on intestinal absorption, a matter which seems not to have been investigated hitherto.

Measurements of intestinal absorption are difficult in the living animal. Blood concentration of substances absorbed from the intestine are the result of rate of absorption, i.e. entry into the blood, and rates of excretion or utilization, i.e. removal from the blood.

Using an in vitro technique allows ready access to both the serosal and mucosal fluid, and therefore it is possible to measure true transport across a concentration gradient. However, it does not have the advantage of in vivo techniques, which allow studies of intestinal absorption under physiologic or near physiologic conditions. There are various technical problems with the in vitro method. The difficulty of adequate oxygenation of the mucosal surface was overcome by everting the intestine, thus exposing the mucosa to the oxygenated medium, and distending it with sufficient fluid to increase the surface area of the sac. The process of
eversion itself may result in considerable injury to the mucosal epithelium. In addition, the amino acid has to pass through the sub-mucosal and the smooth muscle layer before reaching the serosal fluid, and there is some evidence that the muscle itself accumulates amino acid (Crane and Mandelstam, 1960).

As mentioned earlier, the amino acids requirement for broilers depend on age and it is generally considered that they decrease as the animal matures. Nevertheless, this principle cannot be applied to all amino acids. As has been pointed out in the past, the decrease with age does not occur with the same intensity for each amino acid, and recent findings on lysine and methionine illustrate this point. It is not clear, however, whether this conclusion applies to natural or synthetic diets. One of the factors influencing the amino acid requirement is intestinal absorption and this has not been measured before in the broiler chick. The rate of absorption of methionine, cysteine and lysine fell with increasing age of chicks up to 30 days of age. This is in agreement with the National Research Council (1977) who showed that methionine, cysteine and lysine requirement is lower at 3-6 weeks of age when compared with a group at 0-3 weeks of age. Also, Boomgaardt and Baker (1973) came to the same conclusion, finding the requirement for sulphur amino acid decreases with age. However, Graber et al. (1971) found a different result from those of the National Research Council for they found methionine requirement did not exceed 0.63 and 0.70 per cent of diet at two and eight weeks respectively. The difference between those workers is probably due to the conditions of measurement such as the form of methionine supplied and the total protein level in the diet. In general, most workers agree that the methionine, cysteine and lysine
requirement decreases with age. The decrease in amino acid requirements with increasing age is a reflection of the decrease in protein requirement (Bird, 1953; Bornstein, 1970).

The interspecies differences are an interesting aspect of this study, especially when sulphur amino acids are considered. It is known that the availability of sulphur amino acids is one of the most important factors in poultry (broiler) feeding. This is not so in mammalian species. Our results would seem to support this conclusion particularly for cysteine, for the absorption rate for this amino acid was considerably greater in chicks than in rats of comparable age.

The evidence of the results when mixtures of amino acids were used in our work makes it very unlikely that all classes of amino acids are handled by the same transport system in the intestine. In fact there is reasonable kinetic evidence suggesting at least four systems: (a) neutral, (b) basic, (c) acidic amino acid, and (d) imino acid (Wiseman, 1968). The degree to which the specificities of these systems overlap is not clear and further careful studies on this point are necessary. However, the data available on this experiment indicates that neutral and acidic amino acids have very similar kinetic properties, so that considerations of possible specific effect of amino acid charge per se on these properties seems reasonable. Also, this was in contrast to the effect of the basic and acidic amino acid. This explains the competition between these groups for the common carrier and will lead the form of inhibition. It is not known yet if the acidic amino acid is transferred across the brush border membrane by a carrier or on their own. However, our results showed clearly the form of inhibition between
acidic amino acid on one hand and those of basic and neutral amino acid on the other hand. Also, groups of neutral and basic amino acids were reduced in their rate of absorption when mixed together in comparison to each one individually. This is in agreement with Hagihira et al (1961) who found that methionine inhibited uptake of lysine by rings of hamster intestine. In conclusion, mammals and avians have a similar pattern of absorption and inhibition if neutral and basic amino acids were used in the mixed form. Also Chez et al (1971) suggested that lysine counted as a primary user of basic amino acid when both neutral and basic are together. This kind of competition and inhibition between neutral and amino acid was found also in our experiments when different ages were tested for this study. Conclusion may be drawn that competition and inhibition apply to all ages of chicks. Further studies may make possible a distinction between such effect and ones due to specific structural differences among the transport sites themselves.

The results of all the rates of intestinal absorption for methionine in chicks showed high absorption rates when glucose was absent from the perfusion medium, while a decrease was noted when glucose was present. This is in agreement with Newey et al (1970) who demonstrated that the intestinal transfer of proline and methionine into everted gut sacs from rats was increased when glucose was initially absent from the medium. This result in chicks means that avians and mammals have similar patterns of competition for a common carrier during absorption. No similar work has been done before in broiler chicks. It would be worthwhile studying other sugar forms of inhibition in chicks. Also the rate of absorption of sugar in chick intestine may be a factor for further studies.
However, glucose was included in all other experiments in the studies to create a situation more analogous to an *in vivo* condition. The explanation for the increased transfer of methionine when glucose was absent may be a more efficient transfer mechanism, the permeability change in some unrelated to the transfer mechanism or more energy available for that particular mechanism, and finally less competition for a common carrier to that mechanism. Also it is believed that feed intake is in part controlled by the amount of glucose in the blood; however, it is not clear if the amount of glucose in the diet could play an important part in amino acid requirements, but it is clear that amino acid rate of absorption showed a clear indication that it was higher when glucose is absent.

There have been few reports on the effect of antibiotics on the upper digestive tract of broilers. Franci *et al* (1972) showed that chicks fed low-level oxytetracyclin had significantly shorter but heavier small intestine at four weeks of age. On the other hand, Gordon (1952) and Pepper *et al* (1953) found that chicks given penicillin or aureomycin had a lower intestine weight and presumably a thinner wall. Thus, the nature of the antibiotic used would seem to be important.

Braude *et al* (1959) have shown no evidence for a consistent reduction in numbers of intestinal micro-organisms in chicks given antibiotics, so that it is unlikely that the lower weight of the intestine is an indirect result of a lesser microbial burden.

Finally, it is suggested in our result that as antibiotics had no effect on the rate of absorption, and it seems that if they are a growth-stimulating factor this must be related to a reduction in the
degree of infection, which could be controlled by these compounds and which result in a healthier animal.

In these studies we have measured the rate of absorption per unit weight of dry defatted tissue and have also calculated the concentration gradient as a ratio for the concentration of the amino acid on the serosal side: the concentration on the mucosal side. In both species the pattern of change found with age was that as the absorption rate decreased the concentration gradient rose. This was true also for lysine and cysteine in the chick. The changes should be interpreted as indication that as the concentration gradient rose the energy-dependent process of transportation of the amino acid was unable to move the amino acid against the concentration gradient. The one exception to this general pattern was found with cysteine in the rat, for here both the rate of absorption and the concentration gradient fell together. This may indicate that the process of transportation of cysteine in the rat differs from that of the other amino acids in this species and of cysteine in the chick.

The claimed improvement of feed efficiency with antibiotics in the nutrition of poultry is well known (Briggs, 1952; Bird, 1969). However, Franti (1973) in a subsequent experiment showed that there were no significant differences in the rates at which birds treated with oxytetracyclin converted foodstuffs to body weight compared with controls. In the present experiments it was found that antibiotics reduced the absorption of amino acids by the gut sacs. This result would therefore seem to be in agreement with those experiments in which antibiotics were without effect on growth rate. However, it is to be noted that in our experiments antibiotics were given to the
chicks for only three days and it could well be that this time was insufficient for adaptation of the intestine to occur. In practice antibiotics are, in fact, usually given for at least two weeks.

In the mammal cystine or cysteine is formed from dietary methionine via the trans-sulphuration pathway (Dickerson and Basu, 1976). Two enzymes are involved in this transformation, cystathionine synthase which is responsible for the conversion of L-homocysteine to L-cystathionine, and cystathionase which completes the conversion of L-cystathionine to L-cysteine (Fig. 6.1). In different mammals these hepatic enzymes develop at different rates and this accounts for the fact that cysteine is an essential amino acid in the human body, whereas it is not in the rat. A study of these enzymes in avian development would throw further light on the changes in requirement for cysteine during growth.

Further work in this field could be of interest particularly if an amino acid analyser was used to study the different absorption rates of two or more amino acids present in the mixture. The effects of varying concentrations of the amino acids could also be studied. These results could then be translated into dietary concentrations and a growth study carried out in order to ascertain the relationships between intestinal absorption of amino acids and growth.

The interconversion of methionine to cysteine is a matter for further study in connection with sulphur amino acid requirements.
**FIG. 6.1** Conversion of methionine to cysteine

(After Dickerson and Basu, 1976)
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