M.Sc Thesis

Radiation-induced changes in amino acid metabolism following partial-body irradiation in the human.

DECLARATION

I hereby declare that the following thesis is based on research carried out by me, that the thesis is my own composition, and that it has not previously been presented for a Higher Degree.

CERTIFICATE

I certify that Michael George Sturrock, B.Sc., has spent a minimum of seven terms as an external research student of the Faculty of Science of the University of St. Andrews, that he has fulfilled the conditions of Ordinance No. 51 of the University Court of St. Andrews and that he is qualified to submit the accompanying thesis in application for the Degree of Master of Science in Pure Science.

Research Supervisor

CAREER

I matriculated in the Queens College, Dundee of the University of St. Andrews in October 1960 and followed a course leading to graduation in June 1963 with the Ordinary Degree of Batchelor of Science. In July 1964, I accepted the post of Assistant Experimental Officer with the U.K.A.E.A. at Chapelcross Works, Annan, Dumfriesshire, and began research into the problems associated with Biochemical Dosimetry. This research was accepted by the Senatus Academicus of the University of St. Andrews as subject matter for this thesis in October 1964. Under the conditions of Ordinance No. 51 of the University Court of St. Andrews, I have spent $2\frac{1}{2}$ years at Chapelcross.

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1. INTRODUCTION

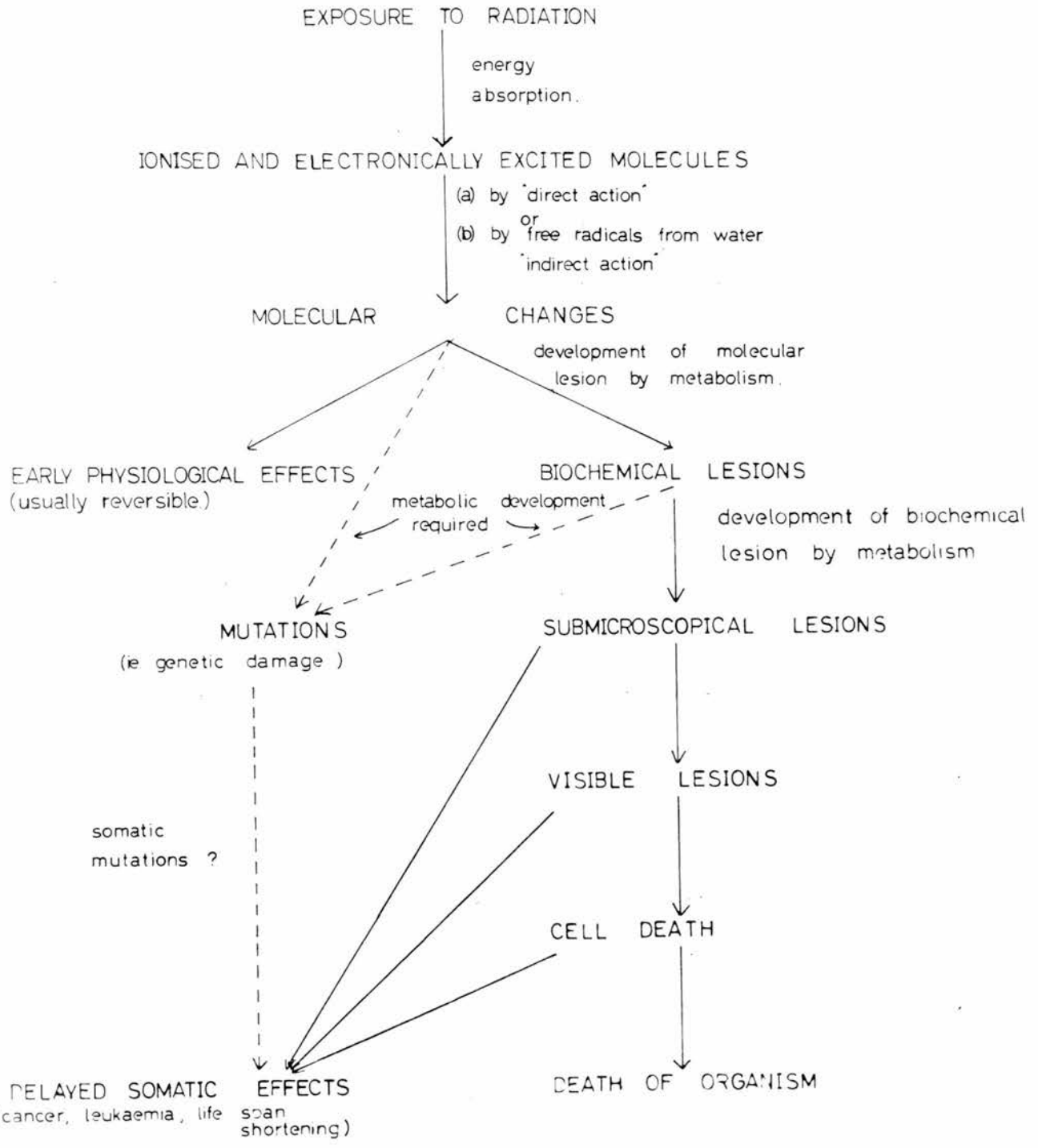
1.1 Biochemical Indicators of Absorbed Dose

Although the use of physical dosimeters is well established as a means of measuring ionising radiation, there are certain disadvantages in the physical approach and it is often not possible to measure, physically, the actual dose received. Physical methods may give an accurate indication of the amount of radiation to which an individual has been exposed, but they do not measure absorbed dose or, in particular, the distribution and biological significance of that dose. Thus a biological indicator fulfilling this purpose is of primary importance. Unfortunately there are limitations of biological indicators of absorbed dose. Chronic low-level exposure is almost impossible to assess because of the repair processes that are functioning continually at a physiological level.

In order to translate the physical phenomenon of ionisation into the ultimate clinical picture of the acute radiation syndrome it is necessary to consider each stage in the sequence of events leading to the response of the body as a whole. Thus, the biochemical changes induced by ionising radiation in living tissues must be viewed at the level of cellular activity.

Fig. 1

THE STEPWISE DEVELOPMENT OF RADIATION INJURY



1.2 Mode of Action of Ionising Radiation on Living Systems

It is pertinent to consider the sequence of events that occur within a cell exposed to ionising radiation. A summary of these effects is shown in Fig. 1. Ionising radiation is a form of energy and in order to have an effect on a living or non-living system it must be absorbed. It is apparent that this absorbed energy induces change at a molecular level, and these changes occur within a micro-second of exposure to radiation. There would appear to be two main mechanisms which, in living systems, cannot be separated.

(i) Direct Action

Molecular damage occurring in the molecule where the energy is absorbed. That is to say, the molecule undergoing change itself becomes ionised or excited by the passage through it of radiation.

(ii) Indirect Action

Here highly reactive free radicals or hydrated electrons react with cell constituents. The interaction of these radicals with important intracellular molecules, such as enzymes, results in the primary biochemical lesion.

Again this is an extremely rapid process which is complete within seconds of the production of the free radicals.

As a result of the continuing metabolism of the irradiated tissues, and provided that a sufficient number of cells are damaged, the primary biochemical injury is translated into a biochemical lesion capable of measurement. As Gray stated (1951) "metabolism plays an essential role in the development of the radiation injury."

It is important to emphasise that the appearance of the biochemical lesion may be delayed for hours or even days after the initial events, but it is only at this point, in time, that biochemical changes can be measured in an attempt to estimate the degree of damage caused by the irradiation.

Subsequent normal metabolic processes are alleged to be responsible for the development of a demonstrable lesion at the anatomical level.

1.3 Biochemical changes following exposure to Ionising Radiation

Considerable information is accumulating on the biochemical changes which follow irradiation but most of this information has been derived from animal experiments,

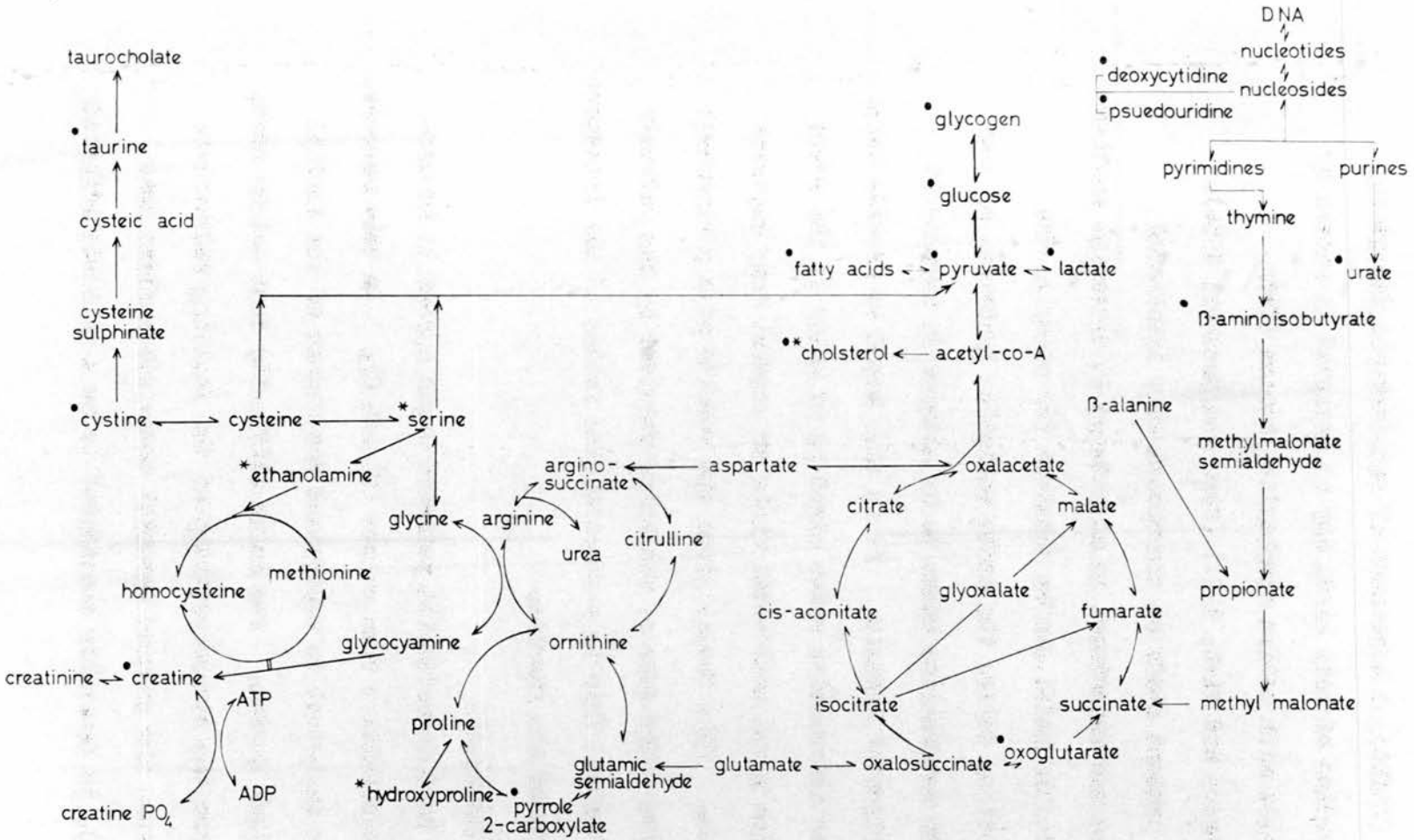
usually following high doses of total body irradiation.

While certain metabolites such as 5-hydroxyindole acetic acid have been shown to be excreted in excess following irradiation in several species, e.g. rat, rabbit and frog (Deanovic, Supek and Randic, 1963, Renson and Fischer, 1959, Brinkman and Veninga, 1961) the magnitude of the excretion and also its variation with dose have both been shown to be markedly influenced by the species, sex and even strain of experimental animal used. This is also true for other metabolites so that the interpretation of the results of animal experiments with a view to understanding radiation injury in man must be done with caution.

A survey of the literature reveals that many metabolites are allegedly altered after irradiation, but the information on the accidently exposed human is limited and disappointing. Therefore it appeared to be of value to undertake a study of certain metabolites in urine and plasma samples obtained from humans who had been exposed to ionising radiation. Occurrences of accidental exposure to radiation are very rare, and consequently studies were confined to therapeutically irradiated patients undergoing treatment in Edinburgh Western General Hospital.

Imbalance of protein metabolism, and hence amino acid metabolism, is believed to occur within hours of exposure to radiation (Dale, Davies and Gilbert, 1949) (Barron, Ambrose and Johnson, 1955) (Katz and Hasterlik, 1955) (Gjessing and Warren, 1961) (Miyazaki, 1963) (Podiltschak et al, 1964) (Ganis, Hendrickson and Howland, 1965). If this is so, then an investigation of urinary and plasma amino acids would be of value. Excretion of amino acids reflects the net result of the numerous chemical reactions which are part of the processes of growth and repair in living tissues.

Studies on accidently irradiated humans have been reported (Kurchara, Rubini and Hempelman, 1961, Wald and Thoma, 1961, Gjessing and Warren, 1961) but in general have suffered from a lack of any control information and from considerable difficulties in assessing the exact dosage. Similarly, while dosage is known with accuracy in therapeutically irradiated humans, it is important to stress that, in studies involving partial body exposure, the sex of the patient often determines the site irradiated and dosage and irradiation technique are frequently determined by site. In addition, the presence or absence of active neoplastic disease is often an important uncontrolled variable; e.g. β-Aminoisobutyric



RELATION BETWEEN MAJOR METABOLIC ROUTES ALLEGED TO BE ALTERED BY RADIATION.

• signifies an increased amount. * signifies a decreased amount.

Fig. 2

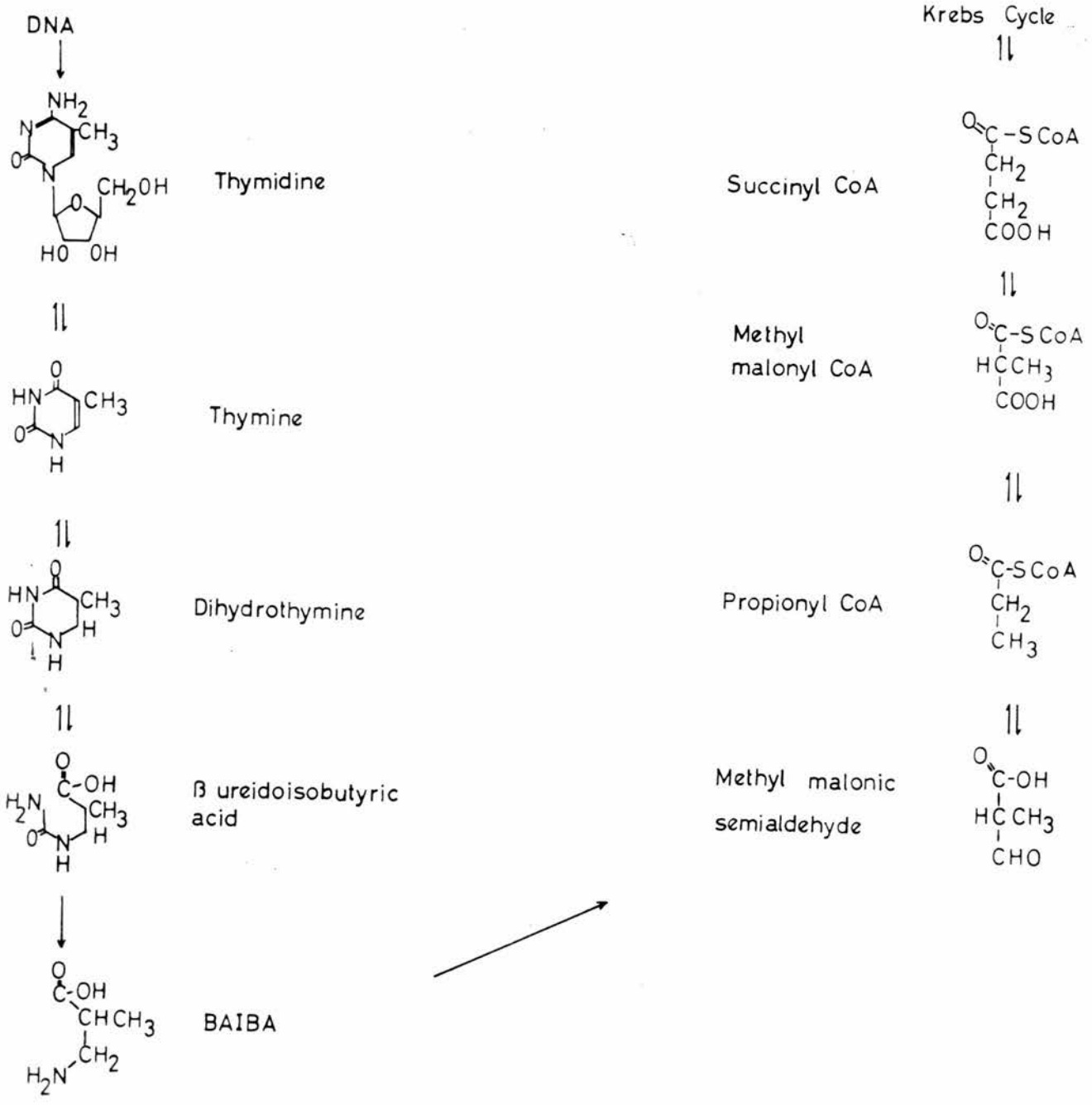
Acid (BAIBA), a substance of considerable importance in studies of this kind, may be excreted in excess by patients with active neoplastic disease (Fink, Henderson and Fink, 1951) (Pare and Sandler, 1954). This present study on therapeutically irradiated humans was undertaken in an attempt to determine whether a suitable model can be obtained for studying the excretion, during the acute radiation syndrome, of some of the metabolites known to be altered in irradiated experimental animals. It is also hoped to provide background information which might be of value in the event of high level accidental whole or partial body exposure in man. This thesis gives the results of a preliminary survey. The dose of radiation received by the patients was low and this is a complicating factor in the interpretation of the results.

1.4 DNA Metabolism

Certain metabolic pathways would appear to be more radio-sensitive than others (Figure 2). In this respect, it is important to understand the nature of the initial chemical lesion. The basic difficulty that arises here, is from the non-selectivity of the ionising radiations. However, the genetic material desoxyribonucleic acid (DNA), is generally considered to be a molecule affected

Fig. 3

METABOLISM OF BAIBA



by the primary lesion (Butler, 1959) (Ord and Stocken, 1958). Subsequent metabolism is thus affected and a study of one catabolic pathway involving BAIBA has been made.

It is reported that irradiation inhibits DNA synthesis as a result of decreased phosphorylation of nucleotides (Creasey and Stocken, 1959). Other authors report excessive production of desoxyribonucleases (Kowlessar, Altman and Hempelmann, 1954), resulting in the increased excretion of uric acid (Kolousek and Dienstbier, 1962, Baker and Hunter, 1961), deoxycitidine (Haley, Flesher and Komesu, 1958) and pseudouridine (Hughes, 1958). The increased availability of nucleotides results in the excessive formation of purine and pyrimidine bases. One of these bases, thymine, is catabolised in the first instance by reduction of the pyrimidine ring to dihydrothymine, followed by ring cleavage to β-Ureidoisobutyric acid and subsequent decomposition to BAIBA. BAIBA is then either excreted by the kidneys or is subsequently transaminated, to methyl malonic acid semialdehyde. This is degraded via succinic acid and the Krebs cycle. The metabolic pathway is summarised in Figure 3. The main site of BAIBA metabolism is thought to be the liver but animal

experiments, at present being carried out, would indicate that the kidney may well play an important role in BAIBA metabolism. (Smith and Chapman, unpublished) Observations on low and high excretors tend to confirm this (Smith and Sturrock, unpublished).

Kretchmar and Phipps (1960); Rubini, Cronkite, Bond and Fliedner (1959) reported that increased urinary excretion of BAIBA occurred after accidental exposure to radiation and Rubini et al (1959) suggested that the dose-dependant response obtained could be used as an index of radiation exposure. Obviously, BAIBA is worthy of further consideration.

1.5 Sulphur Metabolism

The effect of ionising radiation on the metabolism of the sulphur containing amino acids, cystine, cysteic acid and in particular, taurine, a derivative of cysteic acid, was also investigated. The sulphur atom has long been recognised as occupying a key position in both radiation injury and chemical protection against this injury.

Alteration of the normal excretion pattern after accidental high level or therapeutic exposure has been described (Katz and Hasterlik, 1955; Ganis, Hendrickson and Howland, 1965; Langenderff, Melching and Streffer, 1963; Kay and Entenman, 1959). Taurine metabolism is

well documented and confirmatory evidence of hypertaurinuria following X-irradiation is reported in other species (Stern and Stim, 1959; Angel and Noonan, 1961; Bigwood and Soupart, 1962). Both man and rat would appear to show early increases after exposure, but the origin of excess taurine is still under investigation.

This excessive excretion of the sulphur containing derivatives of amino acids, and in particular taurine, is of especial interest. It may be possible that excessive urinary excretion of taurine, which is considered to occur in the absence of a general amino aciduria, is a specific manifestation of radiation damage and therefore of diagnostic value. In addition, since taurine is an end-product of sulphydryl oxidation (Eldjarn, 1954) its excessive excretion may be indicative of damage to sulphydryl groups, which are known to be liable to X-irradiation (Barron, Dickman, Muntz and Singer, 1949).

Hence a study of taurine, and related amino acids, was carried out to see if there was a dose-dependant relationship in connection with increased excretion following X-irradiation.

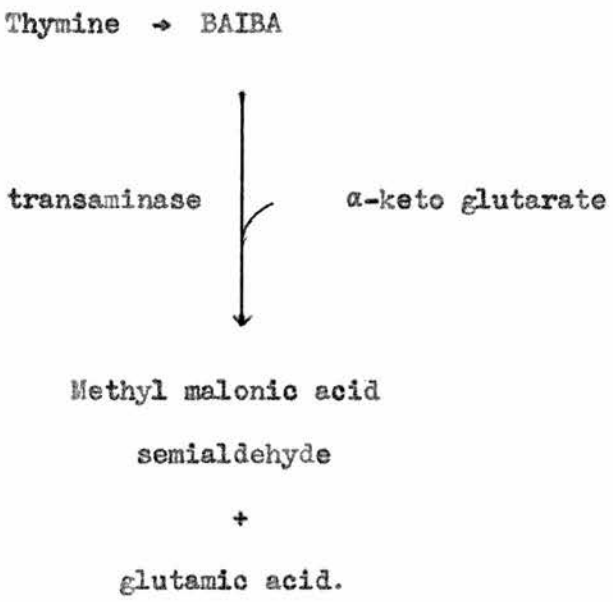
1.6 Other Metabolites

Further investigations included serine and its degradation, by decarboxylation, product ethanolamine.

Since serine is thought to be a precursor of the sulphur-containing amino acid cystine, investigation of its excretory pattern following irradiation seemed to be of value. Decreased urinary excretion of both serine and ethanolamine has been reported in the human after irradiation (Kretchmar, 1959; Cavalieri, Van Metre and Siversten, 1961).

The excretion pattern of valine was also investigated. It has been reported that there is possibly a direct metabolic pathway between BAIBA and valine via methyl malonic acid semialdehyde, in the pig. (Kupiecki and Coon, 1957). If this is so, and if perhaps a comparable metabolic pathway exists in the human, then, as it is generally accepted that there is an increase in BAIBA excretion following X-irradiation, it is reasonable to assume that a variation in the excretory pattern of valine might be demonstrable in the human following X-irradiation.

In conjunction with the valine studies, glutamic acid was also investigated, from a consideration of its metabolic relationship to BAIBA.



1.7 Summary

In summary, an investigation into the changes in the excretion pattern of several amino acids in urine and plasma samples was undertaken in patients exposed to therapeutic doses of X-irradiation.

2. CLINICAL DATA

2.1 Types of Case Studied:-

In order to overcome some of the aforementioned disadvantages inherent in this type of study, observations were restricted to changes following a single acute exposure in males. It was necessary to accept the limitations of partial body exposure with the possibilities of abscopal effects, and to limit the observations to a maximum of 96 hours post-irradiation in order to avoid undue interference with treatment schedules.

The patients studied were either cases of ankylosing spondylitis (a non malignant condition) undergoing radiotherapy to the whole spine and sacroiliac joints, or cases receiving radiotherapy to the whole abdomen following orchidectomy for testicular neoplasm. In this latter group all patients with clinically demonstrable metastases were excluded. Irradiation was therefore being administered on a prophylatic basis, and this group can be considered to approximate closely, to normal healthy males.

The abdomen is a peculiarly radiosensitive part of the body and it was felt that it was desirable to have a second group of spondylitic patients for comparison, though it is not feasible to equate dosage between the

two groups.

All cases were in-patients in the radiotherapy wards for the period of study. No attempt was made to control activity; diet was restricted solely in terms of foods said to be rich in 5-hydroxytryptophan, such as bananas. No drugs whatsoever were given to combat radiation sickness, since they might interfere with the metabolic systems under consideration. On the day of irradiation a light breakfast was given at 6.30 a.m., irradiation was carried out at 10 a.m. Since most patients experienced some radiation sickness, lunch was routinely omitted, although patients were encouraged to drink enough to ensure an adequate rate of urine flow.

Many of the patients studied, developed a mild form of the acute radiation syndrome with anorexia, nausea, vomiting and lassitude. On the basis of this symptomatology they would appear to correspond to a level of whole body exposure of about 75-100 rad.

2.2 Irradiation Techniques

Abdominal irradiation was carried out on a 4 MeV linear accelerator through two parallel opposed fields designed in each case to include the whole abdomen from the level of the xyphisternum to the root of the penis, and measuring, on average 34 × 18 cm. It is standard

practice to shield the kidneys during abdominal irradiation but the kidney shields were omitted for this initial treatment.

A dose of 150 rads., calculated at the mid-thickness of the abdomen was delivered in every case at a dose rate of 75-100 rads./minute at an F.S.D. of 120 cm. on average. Time zero was taken as the commencement of irradiation which was complete within four or five minutes.

Spinal irradiation was carried out on apparatus operating at 250 kV X-rays (Thoraeus I filter, h.v.l. 2.7 mm. Cu.). Irradiation was delivered through three posteriorly placed fields along the spinal axis, measuring 7.5 cm. in width and totalling on average 55 cm. in length. The sacro-iliac joints were irradiated through a 15 × 10 cm. posteriorly placed field, the 15 cm. axis being transverse. Dosage which varied between 100 and 300 rads., measured on the skin surface inclusive of back scatter, was delivered at an average dose rate of 67 rads./min. at 50 cm. F.S.D. Again the commencement of irradiation was taken as time zero, though in this instance the overall treatment time varied up to 20 minutes.

2.3 Collection of Specimens

(i) Urine:- This was collected directly into plastic

bottles and immediately refrigerated at 4°C until the 24 hour collection was complete. The total volume was then measured and aliquots were deep frozen at -20°C, without preservative, until analysed. Urine was collected in 24 hour aliquots for the 24-48 hours preceding irradiation and up to 96 hours post-irradiation. In later studies, urine collections were time-fractionated on a basis of 2, 2, 4 and 16 hours and stored and analysed separately.

(ii) Blood:- This was collected by venepuncture, coagulation being prevented by dry heparin (500 units/ 20 ml.). Usually three control blood samples were withdrawn in the 24-48 period preceding irradiation and further samples were taken at 2, 4, 8, 12, 24, 36, 48, 72 and 96 hours post-irradiation. In all tables the day of irradiation is referred to as day 1 and the days following are numbered consecutively. The times of withdrawal of blood samples are indicated as hours after irradiation e.g. X + 48 hrs. The plasma was separated immediately from the cells without haemolysis and the two fractions retained at -20°C.

Subsequently, when the investigations were concentrated on thymine loading "function tests" in

an attempt to obtain a suitable model for the study of BAIBA metabolism, a slightly modified sampling scheme was adopted, incorporating "time-fractionated" urine sampling. The following table describes a typical programme for the collection of specimens during "thymine loading" experiments.

Table 2.1 Programme for Collection of Samples

Thymine Loaded; Seminoma Testis 150 rad.

URINE		BLOOD	
Sample No.	Time Interval	Sample No.	Time Interval
1	24 hrs. Control	1 + 2	Control
* 2	2 hrs.)	3	1 hr. post thymine
3	2 hrs.) Controls	4	3 hrs. post thymine
4	4 hrs.) Post-thymine	5	6 hrs. post thymine
5	16 hrs.)		
* 6	0- 2 hrs. X-Irradiation	6	1 hr. post thymine + 1 hr.
7	2- 4 hrs.	7	3 hrs. post thymine + 3 hrs.
8	4- 8 hrs.		
9	8-24 hrs.		
*10	24-26 hrs.	8	1 hr. post thymine + 25 hrs.
11	26-28 hrs.	9	3 hrs. post thymine + 27 hrs.
12	28-32 hrs.		
13	32-48 hrs.		
*14	48-50 hrs.	10	1 hr. post thymine + 49 hrs.
15	50-52 hrs.	11	3 hrs. post thymine + 51 hrs.
16	52-56 hrs.		
17	56-72 hrs.		

*500 mg Thymine given orally

One major problem in this type of study is that the supply of patients is obviously limited. In any one year, one might expect about 12-15 patients, but because of the statistical basis upon which the patients present themselves, the analytical programme appears to be erratic.

3. ANALYTICAL METHODS

3.1 General Method

The samples obtained from the patients receiving radiotherapy, both urines and plasmas, were analysed using a standard Technicon Automatic Amino Acid Analyser, developed from the techniques of Spackman, Stein and Moore (1958), Hamilton (1958) and Piez and Morris (1960). This system permits higher resolution and greater reproductibility than paper chromatographic methods. The Technicon apparatus consists basically of two systems:-

(i) The Chromatographic System

(ii) The Analytical System

3.2 The Chromatographic System

Initially, in this series of investigations, the chromatographic system consisted of a single adsorption column of a Technicon resin manufactured under the name of "Chromobeads". This is a type A, 8% cross-linked, sulphonated, polystyrene resin, having spherical particles of a specified diameter of 16-18 microns. That is, 8% divinylbenzene has been added to the monomer before it polymerises. This results in more active sites and gives improved resolving power and speed of elution.

A 15-hour run with a flow rate of 0.75 ml/min. and a

pumping pressure of 250 lb/in^2 using a Milton-Roy positive displacement micro-pump was found to be quite adequate for the peak resolution of the majority of the amino acids commonly found in urine and plasma. A suitable buffer gradient was obtained using the Technicon development of the technique of Peterson and Sober (1959). The nine-chamber Technicon Autograd device was used for simultaneously producing a smooth pH gradient between 2.875 and 5.00, and a continuous sodium ion gradient between 0.2M and 0.8M. Citrate buffer was employed and was made up in 10 litre portions from a "stock" solution and stored at 4°C in a fridge. Later the buffer solutions were treated with a preservative N-caprylic acid, which obviated fridge storage. Buffer and autograd make-up are as reported in the Technicon manual (1960).

Subsequently a second adsorption column of identical resin was incorporated, and a simple Two-Column System for the analysis of urine and plasma samples was developed. The volume of the buffers in the autograd was doubled without changing the pH gradient of the original equipment. A single micro-pump working at double pressure was used to pump the buffer from this single source through the two columns simultaneously. This meant that the flow rate through the two columns must be the same and hence

accurate balancing and care when packing the columns was essential. The advantage of this system, apart from the obvious economy of equipment, is that two different samples can be analysed in parallel simultaneously, an expedient which enables the operator to compare a normal sample with an abnormal one, under conditions which are nearly identical; in this particular case, of course, pre-irradiation samples can be compared with post-irradiation ones simultaneously. Under this system the micro-pump was operated at a flow rate of 1.5 ml/min. giving 0.75 ml/min. through each column when accurately balanced.

Fibre glass insulated water jackets were connected in parallel and then the columns were maintained at identical temperatures by the thermo regulated pump (Haake Bath) furnished with the original equipment.

3.3 The Analytical System

The column effluent was automatically reacted with ninhydrin reagent and the colour yield at 570 mμ and with a 15 mm light path was recorded using standard Technicon equipment. The respective absorption peaks were determined by the height × width (H × W) method as described by Spackman, Stein and Moore (1958) and expressed initially in terms of norleucine colour equivalents. The correction

factor for colour yield of each of the common amino acids as compared with norleucine was obtained from Hamilton's values (1963).

3.4 Characteristics of the Ninhydrin Reaction

The colour reagent used was ninhydrin (triketohydrindene hydrate) first developed by Moore and Stein (1948). The reaction of ninhydrin with NH_2 groups to give a blue-coloured pigment diketohydrindylidene-diketohydrindamine has been untilised as the basis for photometric determination of amino acids and related compounds.

The reaction is carried out at pH 5.0, the ninhydrin acetate buffer being of sufficient concentration to overcome the variable buffer from the chromatographic system, and at 95°C. The absorption maximum of the blue product is at 570 mμ.

The ninhydrin reagent possesses the advantage of being stable, under nitrogen in a darkened container, and for routine use can be stored in this way for a month or more. It was made up, one week's supply (10 litres) at a time and stored under 'white spot' nitrogen in a blackened air-tight container. The composition of the stock ninhydrin is given in the following table:-

Table 3.1 Composition of Ninhydrin Reagent

Hydrindantin	3.76	g.	Stage 1
Ninhydrin	50	g.	
Methyl Cellosolve	1625	c.c.	
Methyl Cellosolve	2875	c.c.	Stage 2
Water	3760	c.c.	
Methyl Cellosolve	875	c.c.	Stage 3
Acetate Buffer (pH 5.5)	875	c.c.	

During Stage 2 of the ninhydrin make-up, the reagent was mixed with 200 mesh Zeo Karb 225 cationic resin (H^+form).

This cut down ammonia contamination of the ninhydrin solution. The reagent was then filtered off from the resin before the addition of the acetate buffer.

The color yields are rendered fully reproducible by the incorporation of hydrindantin in the reagent solution to eliminate oxidative side reactions. Also excess hydrindantin is added so that the reaction is of the 1st Order

i.e. Color $\propto$ (amino acid),

and not Color $\propto$ (amino acid) (hydrindantin).

Although the color yield from a given amino acid is constant, the different amino acids do not all give the

same percentage yield of the blue product. But this factor does not prevent the accurate use of the method in chromatographic work in those cases in which the individual amino acids are separated by the fractionation of a sample on a column, since the ninhydrin reaction can be made to give quantitative values by use of a factor appropriate to the amino acid in question.

In addition to internal standards, external norleucine standards (0.05 mM in $\frac{N}{10}$ HCl) were also run for assessment of stability of the analytical system, and in particular, the condition of the proportioning pump tubing, and the ninhydrin reagent.

3.5 General Procedure

The procedure for a typical analytical run of a urine sample was as follows. The chromobead column was first regenerated after the previous "run". The basic regeneration procedure consists of two stages.

(i) Pump 0.2N sodium hydroxide for half an hour, or at least until eluant comes through alkaline.

(ii) Pump citrate buffer pH 2.875 (the initial buffer) until eluant comes through acid. This is a period of about one hour, kept minimal to obviate BAIBA peak and base-line ammonia rise

coincidence.

The aliquot of the thoroughly mixed sample, normally $\frac{1}{5000}$ of the total daily urinary output, was acidified with 1-2 drops $\frac{N}{10}$ HCl (providing for adjustment below the initial buffer pH) and accurately applied to the 130 x 0.63 cm column with a micro-pipette. This was followed by 0.1 μM of an internal standard (Norleucine in $\frac{N}{10}$ HCl). The sample was packed onto the column with nitrogen under pressure (30-40 lb/in^2). The column was then topped up with the initial buffer and the time switches set to give a 15 hour run.

The procedure for plasma samples varied only in the preparation of the sample for loading onto the columns. The plasma proteins must first be precipitated, to avoid column blockage, and this was achieved using a slightly modified version of Hamilton's Sulphosalicylic Acid technique. The hazards in deproteinisation are:-

(i) Co-precipitation of amino acids.

(ii) Hydrolysis of amino acids due to too strong an acid.

To 2 ml of a plasma sample was added an equal volume of 10% sulphosalicylic acid, and the precipitated proteins were removed by centrifugation after allowing sample to stand for a minimum of 2 hours to ensure complete

precipitation. 2 ml of the supernatant was then added to the column (i.e. 1 ml of plasma) and the sample packed down by nitrogen under pressure as before.

Other methods for protein precipitation were investigated, involving denaturation by alcohol solutions of varying strengths, and ultrafiltration. The former method resulted in incomplete precipitation. Ultrafiltration provided a suitable standard for comparison of the sulphosalicylic acid method, in order to elucidate if:-

(i) there was any loss of free amino acids

(ii) any interference of chromatogram by the sulphosalicylic acid.

3.6 Modified Techniques

As the programme proceeded, interest became largely centred upon BAIBA, as a general study of the amino acid pattern at low levels associated with therapy was proving to be of limited value. Consequently, a special 3-column chromatographic system for the rapid analysis of BAIBA was introduced.

The major difference from the normal Technicon system was that a single buffer (pH 3.8) was used, thus doing away with the 9-chamber autograd. (Dymond; to be published). This made the method quicker, simpler, more

economical, and nullified any inaccuracies in autograd technique and buffer make-up. Three columns were set up on a frame-work of Lablox, and heated in parallel from a single Haake Bath. The temperature was increased to 72°C, 4°C above the normal Technicon working temperature. This gave improved resolution. The columns were accurately balanced and using this system a $2\frac{1}{2}$-3 hour run at 1 ml/min. and pumping pressure of 250 lb/in^2 was suitable for rapid BAIBA determination.

Internal and External Norleucine standards were utilised as with previous system. A single micro-pump working at a total flow rate of 2 ml/min. pumped the buffer from a single source through two columns simultaneously. At the same time a second pump (a D.C.L. micro-pump) passed 0.4N sodium hydroxide through the third column, in the first step of column regeneration. The overall working system is simply shown in the following table:-

Table 3.2 Analytical Programme for BAIBA Determination

Column I	Column II	Column III
1. Load and Run (3.8 buffer)	3.8 buffer	0.4N NaOH
2. NaOH	Load and Run	3.8 buffer
3. 3.8 buffer	NaOH	Load and Run
4. Load and Run	3.8 buffer	NaOH

Using this systematic scheme, 3-4 BAIBA determinations could be obtained in one day. Further modifications involved the use of short columns (50 × 0.63 cms) which gave faster regeneration and approximately 2 hour chromatographic runs for BAIBA resolution, giving about 5-6 BAIBA determinations per day.

3.7 Concept of Thymine Loading

There is one major disadvantage in the use of BAIBA as a biochemical indicator of radiation damage, in that its accurate detection and determination in normal urine samples is difficult. The reason for this, apart from its very low color yield with ninhydrin in comparison to most of the other naturally occurring amino acids, is that there is a very low plasma concentration of BAIBA. Thus although a low renal threshold exists in humans (Armstrong et al, 1963), only small amounts are excreted. There are exceptions. A small percentage of the population in this country are found to normally excrete increased amounts of BAIBA in the urine, and are termed 'high-excretors'. They are believed to lack the necessary enzymatic system for the breakdown of BAIBA which consequently accumulates, and is subsequently excreted in increased amounts (Crumpler, Dent, Harris and Westhall, 1951). This is said to be an hereditary defect (Harris, 1953; Calchi-Novati et al, 1954;

Gartler, 1956), and this class of subject provides an unique opportunity to investigate BAIBA metabolism. However, the majority of people normally excrete low concentrations of BAIBA, and to overcome the low renal threshold effect on its excretion, a series of experiments were carried out in which the patients were initially "loaded" with thymine, administered orally. Since thymine is a major precursor of BAIBA (Fink, Henderson and Fink, 1951), BAIBA concentrations were increased after loading and hence BAIBA was more readily detectable. As will be discussed in a later section, the catabolism of BAIBA is a limited reaction in the human and it was postulated that inhibition of the enzyme reactions by irradiation would manifest itself as an elevated plasma BAIBA concentration above the normal and hence an increased urinary output of BAIBA.

One purpose of this thesis is to emphasise the value of loading with a metabolic in order to saturate the metabolic pathway. This technique is widely used in clinical biochemistry to demonstrate early or mild forms of disease and it is suggested that sub-clinical changes due to low-level irradiation may be demonstrable by such techniques.

4. RESULTS AND DISCUSSION : PRELIMINARY SURVEY

4.1 General Survey

Initially a general study of the changes in the amino acid pattern in urine samples obtained from patients receiving small therapeutic doses of X-irradiation (150 rad or 300 rad partial body) was undertaken. Extensive plasma studies were omitted meantime due to the limitation of lengthy analysis time on the Auto Technicon, which allowed only five chromatographic runs per week under the standard system. The preliminary 15-20 hour chromatograms contained approximately 40-50 peaks, corresponding to ninhydrin reactive compounds in the urine, some 30 of which were positively identified by spiking samples with standard amino acid solutions. With the limited equipment available, it was altogether too great a task to investigate the radiosensitivity of each one of these peaks. Accordingly, from a consideration of the initial chromatograms and a survey of the literature, 6 amino acids and related compounds were selected for intensive study.

Tables 4.1-4.8 show the results of the preliminary survey which involved the sulphur-containing metabolites Cysteic Acid, Taurine and Cystine; Serine and Ethanolamine; and BAIBA. Table 4.9 shows a summary of the changes observed in these urinary metabolites.

Table 4.1 Urinary Serine (μM/24 hr)

Subject	PRE	Day 1	Day 2	Day 3	Day 4
Testicular Tumours					
J. McC.	730	391	650	800	-
R. W. C.	300	370	460	460	590
R. S.	460	350	417	550	880
J. T. M.	989	412	737	917	-
R. S. C.	445	473	700	708	-
J. R. N.	404	516	416	305	-
J. S.	318	301	582	775	180
A. M.	601	403	549	773	-
J. R. B.	420	501	438	348	262
A. B.	234	433	315	170	160
N. B. B.	496	371	298	235	525
J. D. M.	690	623	505	461	399
D. C. R.	660	588	547	618	634
A. J.	236	121	424	512	412
I. McD.	145	321	400	421	-
O. M. H.	1050	670	1205	1070	-
T. G. W.	420	463	263	560	-
G. S. H.	516	639	753	-	-
D. W.	673	323	684	305	-
D. McK.	556	505	350	-	-
E. D. C.	779	1295	951	1157	-
Ankylosing Spondylitics					
T. C. B.	700	640	-	-	-
W. T.	432	508	585	504	-
W. B.	547	443	592	656	-
M. M.	1140	848	1090	1220	-
M. C.	814	430	1085	1320	-
S. B.	388	724	610	440	650
J. K.	272	290	642	450	825

4.2 Serine

Decreased excretion of free serine from irradiated humans has been reported (Kretchmar, 1959). Following a study of eight workers who were accidently exposed to whole-body irradiation at Oak Ridge in America he reports that free serine in the urine was decreased, even on the first day after exposure, and in one subject, who received a dose of 365 r, serine was apparently absent in the urine, 3 days after exposure. No such precise agreement was observed in the present series.

A statistical survey of Table 4.1 reveals little overall change in the excretion pattern of free serine, but perhaps with a slight tendancy to decreased excretion on day one following X-irradiation exposure, and an increase on days 2 and 3. Eight of the subjects did show a marked decrease but most returned to normal by day 3 or 4. Conversely some showed a rise following exposure, and one patient in particular, namely E.D.C. displayed a very marked increase in urinary serine, with an elevated excretion still present on day 3.

Overall, it would appear that, at these low levels of exposure, there was no definite trend towards either increased or decreased levels of free serine in the urine and, in the case of this particular metabolite, individuals

METABOLIC PATHWAYS OF SERINE AND ETHANOLAMINE

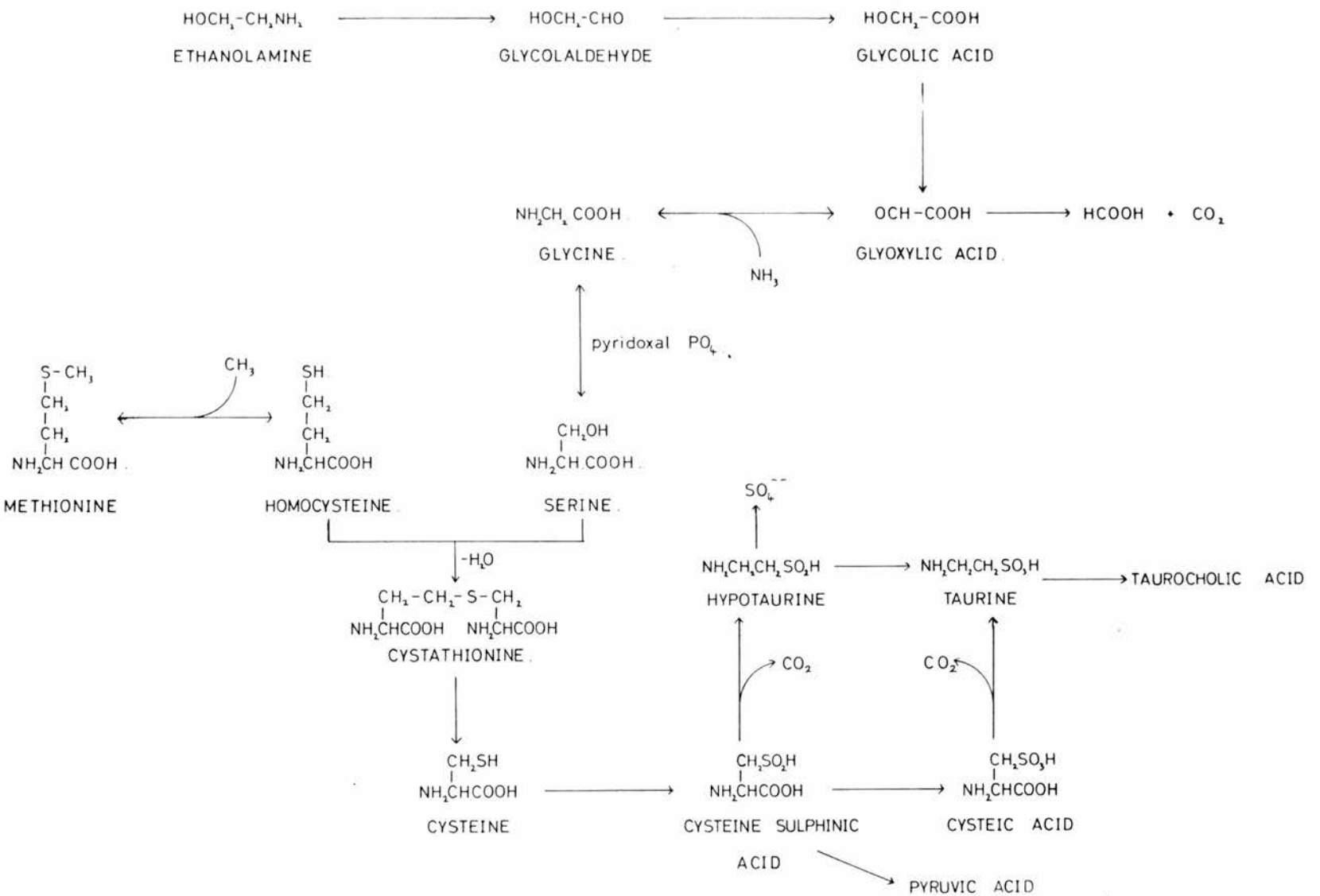


Fig. 4.

Table 4.2 Urinary Ethanolamine (μM/24 hr)

Subject	PRE	Day 1	Day 2	Day 3	Day 4
Testicular Tumours					
J. McC.	293	393	408	424	-
R. W. C.	300	410	453	367	419
R. S.	505	608	287	390	507
J. T. M.	308	510	224	245	-
R. S. C.	434	587	639	486	-
J. R. N.	430	486	627	356	-
J. S.	292	226	284	173	60
A. M.	317	347	337	194	-
J. R. B.	458	233	328	396	173
A. B.	90	135	187	164	122
N. B. B.	208	245	209	224	271
J. D. M.	98	293	334	372	183
D. C. R.	469	630	475	595	446
A. J.	49	175	198	293	311
I. McD.	259	408	414	590	-
O. M. H.	594	537	385	400	-
T. G. W.	258	489	355	462	-
G. S. H.	733	926	866	-	-
D. W.	398	425	667	-	-
D. McK.	-	-	-	-	-
E. D. C.	571	856	714	669	-
Ankylosing Spondylitics					
T. C. B.	405	365	-	-	-
W. T.	860	990	454	534	-
W. B.	282	447	397	314	-
M. M.	777	310	2930	170	250
M. C.	267	443	318	357	-
S. B.	274	930	373	318	481
J. K.	191	268	248	233	261

would appear to display wide variations in response to X-irradiation. In particular, there was no observed difference in response between the patients undergoing treatment for testicular tumours and those for ankylosing spondylitis.

4.3 Ethanolamine

As a result of its metabolic relationship to Serine (see Figure 4), it was considered to be of value to undertake a study of the excretion pattern of ethanolamine following X-irradiation. Decreased excretion of ethanolamine in humans following exposure to whole-body radiation has been reported (Cavalieri, Van Metre and Siversten, 1961). Their findings were reported on the results obtained from patients receiving whole-body radiation treatment for far-advanced neoplastic disease, which was resistant to convential therapy. No plasma sampling was carried out, and therefore renal effects cannot be ruled out. Again, no such indication of reduced urinary ethanolamine following exposure was observed in these studies. In fact, a study of Table 4.2 indicates if anything, perhaps a slight tendency to elevation of this metabolite following X-irradiation. All but four of the twenty-seven patients studied showed a rise at some time following treatment, generally on the first day. One

patient, namely M.M., (a spondylitic) showed a strange response. On the day following treatment, there was a marked drop in the urinary output of ethanolamine, but on the second day after exposure there was a fantastic rise in the level of this metabolite in the urine.

However, overall there was no marked significant change apart from this single patient, but this slight tendency to increased urinary ethanolamine at low levels of exposure may well be a reflection on the prolonged and greatly increased output of ethanolamine observed in the monkey following a lethal dose of 900 rads whole body (Table 6.5a).

Again there was no observed difference in response between seminomas and spondylitics.

4.4 Cystine

Increased excretion of cystine in humans following partial-body exposure to large doses of ionising radiation has been reported (Ganis, Hendrickson and Howland, 1965).

Marked cystinuria has also been reported in cases of liver disease (Walshe, 1953), who further suggested that an increased excretion of cystine is considered to be the most sensitive index of impaired amino acid metabolism.

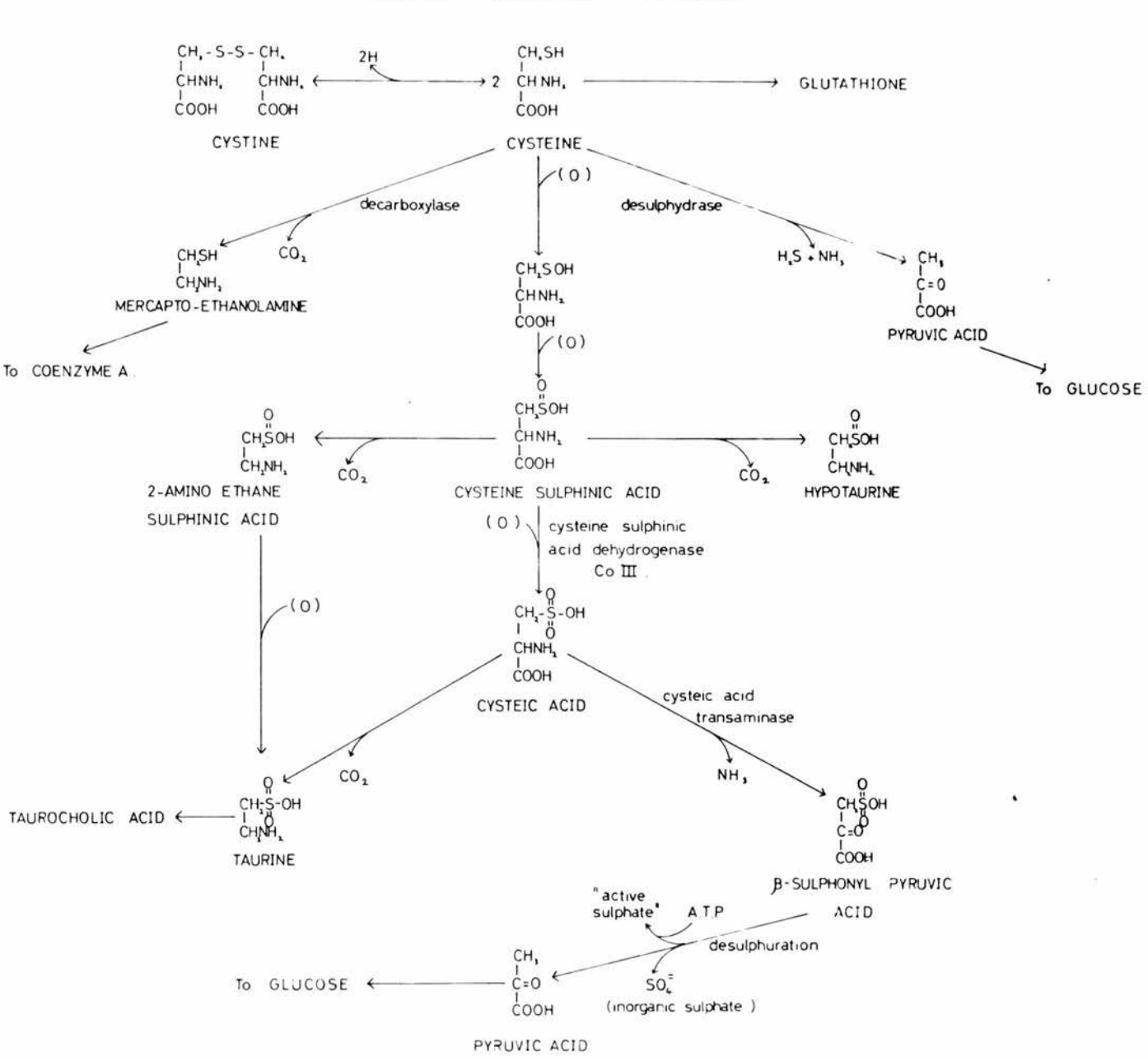

SULPHUR METABOLISM. PATHWAYS
CYSTINE
2H
2
CYSTEINE
GLUTATHIONE
decarboxylase
CO2
MERCAPTO-ETHANOLAMINE
To COENZYME A
(O)
desulphydrase
H2S + NH3
PYRUVIC ACID
To GLUCOSE
2-AMINO ETHANE SULPHINIC ACID
CYSTEINE SULPHINIC ACID
HYPOTAURINE
cysteine sulphinic acid dehydrogenase Co III
CYSTEIC ACID
cysteic acid transaminase
NH3
TAUROCHOLIC ACID
TAURINE
β-SULPHONYL PYRUVIC ACID
"active sulphate"
ATP
desulphuration
SO4=
(inorganic sulphate)
To GLUCOSE
PYRUVIC ACID

Fig. 5

Table 4.3 Urinary Cystine (μM/24 hr)

Subject	PRE	Day 1	Day 2	Day 3	Day 4
Testicular Tumour					
J. McC.	24	31	40	37	-
R. W. C.	25	48	16	29	20
R. S.	69	20	48	41	26
J. T. M.	67	84	40	47	-
R. S. C.	30	37	47	29	-
J. R. N.	18	82	124	64	-
J. S.	47	15	128	22	20
A. M.	69	101	72	34	-
J. R. B.	81	70	51	40	43
A. B.	9	12	8	6	5
N. B. B.	1	12	27	13	31
J. D. M.	28	13	28	12	12
D. C. R.	24	30	38	41	45
A. J.	10	81	114	127	59
I. McD.	53	44	44	81	-
C. M. H.	142	162	176	201	-
T. G. W.	87	99	61	76	-
G. S. H.	16	20	29	-	-
D. W.	95	55	137	37	-
D. McK.	120	56	86	-	-
E. D. C.	73	122	96	114	-
Ankylosing Spondylities					
T. C. B.	70	77	-	-	-
W. T.	29	103	38	31	-
W. B.	71	58	75	66	-
M. M.	110	35	22	13	16
M. C.	31	25	10	16	-
S. B.	50	90	72	82	65
J. K.	41	82	94	103	103

The results here again prove inconclusive (Table 4.3). Days 1 and 2 post-irradiation indicate perhaps a slight trend towards increased urinary cystine in agreement with these authors. But with the exception of subject O.M.H. who normally excreted cystine in the upper normal range, none of the patients showed a rise, after irradiation, above the normal daily range. On the fourth day post-irradiation there appeared to be a drop in the urinary output of this metabolite, but a lack of satistical evidence, only twelve observations for day 4, must be taken into account and this 40% decrease might well be an artificial figure. Obviously the degree of exposure was too low to produce any significant alteration in the urinary output of cystine.

4.5 Taurine - (2 - aminoethane sulphonic acid)

Taurine is the cholic acid conjugate in mammalion bile which forms the bile acid taurocholic acid and it it derived from cystine (see Figure 5). It is a very important product of sulphur-containing amino acid metabolism and is one of the most quantitatively significant constituents of the urine. Hypertaurinuria in animals, following exposure to various doses of X-irradiation has been widely reported (Kay and Entenman, 1954, 1956, 1957; Mefferd and Martens, 1955; Stern and Stim, 1959; Angel

Table 4.5 Changes in Urinary Excretion of Taurine in Human after Irradiation

Patient	Condition	Maximum Rise Above Pre-Irradiation Level (μM/24 hr)	Day of Maximum Rise After Irradiation
J. Mc.C.	Seminoma	-	-
R.W.C.	"	490	+ 2
R.S.	"	436	+ 4
J.T.M.	"	250	+ 1
R.S.C.	"	1470	+ 1
J.R.N.	"	2680	+ 2
J.S.	"	198	+ 2
A.M.	"	1012	+ 3
J.R.B.	"	1035	+ 3
A.B.	"	253	+ 1
N.B.B.	"	104	+ 4
J.D.M.	"	-	-
D.C.R.	"	-	-
A.J.	"	1479	+ 3
I.Mc.D.	"	487	+ 3
O.M.H.	"	-	-
T.G.W.	"	12	+ 2
G.S.H.	"	1548	+ 2
D.W.	"	673	+ 2
D.Mc.K.	"	-	-
E.D.C.	"	-	-
T.C.B.	Spondylitis	117	+ 1
W.T.	"	240	+ 2
W.B.	"	180	+ 2
M.M.	"	320	+ 4
M.C.	"	-	-
S.B.	"	714	+ 4
J.K.	"	592	+ 4

All except D.Mc.K., O.M.H. and J.D.M. showed a rise in urinary Taurine at some point following X-irradiation.

Table 4.4 Urinary Taurine (μM/24 hr)

Subject	PRE	Day 1	Day 2	Day 3	Day 4
Testicular Tumour					
J. McC.	-	-	-	-	-
R. W. C.	430	750	920	890	900
R. S.	414	540	560	525	850
J. T. M.	760	1010	590	630	-
R. S. C.	760	2230	2200	1800	-
J. R. N.	1060	2600	3740	2720	-
J. S.	382	481	580	420	522
A. M.	870	862	1393	1882	1367
J. R. B.	825	854	920	1860	886
A. B.	269	524	401	234	264
N. B. B.	550	572	510	270	654
J. D. M.	390	212	319	382	258
D. C. R.	-	-	-	-	-
A. J.	201	714	1146	1680	1200
I. McD.	916	726	1000	1403	-
O. M. H.	1320	1110	1240	896	-
T. G. W.	448	361	464	335	-
G. S. H.	982	2050	2530	-	-
D. W.	779	780	1452	480	-
D. McK.	1150	845	835	-	-
E. D. C.	-	-	-	-	-
Ankylosing Spondylitics					
T. C. B.	97	214	-	-	-
W. T.	650	860	890	830	-
W. B.	154	207	334	248	-
M. M.	660	831	770	810	980
M. C.	-	-	-	-	-
S. B.	446	643	1050	848	1160
J. K.	168	235	664	580	760

and Noonan, 1961).

It has also been observed in man (Hempelman et al, 1952; Kay and Entenman, 1959; Bigwood and Soupart, 1962).

A study of Tables 4.4 and 4.5 reveals a fairly significant increase in urinary taurine even at the low levels of exposure involved. The mean maximum increased output occurs on the second day following exposure, but there would appear to be a wide individual variation in the time of the highest taurine excretion level (Table 4.5). Elevated urinary levels are still evident on the fourth day. It is reasonable to assume from these observations that there is a definite low level response in the case of this particular metabolite. Since taurine is one of the major end-products of sulphydryl oxidation, (Fromageot, 1947; Eldjarn, 1954; Cavallini, Mondovi and De Marco, 1955; Verly and Koch, 1954) its excessive excretion might suggest an increase in the rate of oxidation of this radical. Barron et al (1949, 1950) have shown sulphydryl-containing enzymes to be especially sensitive to inactivation by X-irradiation. In addition, when given prior to irradiation, cystine (Patt, 1949), glutathione (Chapman et al, 1949) and cysteamine (Bacq, 1953, 1954), protect against radiation death. All these

Table 4.6 Urinary Cysteic Acid (μM/24 hr)

Subject	PRE	Day 1	Day 2	Day 3	Day 4
Testicular Tumour					
J. McC.	88	118	105	25	-
R. W. C.	97	58	115	100	100
R. S.	93	113	76	29	87
J. T. M.	44	70	43	49	-
R. S. C.	120	120	180	114	-
J. R. N.	119	127	154	130	-
J. S.	90	106	132	77	32
A. M.	122	129	114	100	-
J. R. B.	110	80	99	105	66
A. B.	79	138	113	58	34
N. B. B.	70	57	57	40	54
J. D. M.	131	89	87	121	53
D. C. R.	81	29	22	68	75
A. J.	60	58	80	70	66
I. McD.	81	75	73	103	-
O. M. H.	152	128	162	122	-
T. G. W.	114	124	77	108	-
G. S. H.	116	133	132	-	-
D. W.	93	40	55	26	-
D. McK.	90	75	60	-	-
E. D. C.	132	216	152	147	-
Ankylosing Spondylitics					
T. C. B.	65	76	-	-	-
W. T.	82	120	137	119	-
W. B.	121	124	146	104	-
M. M.	170	132	109	107	123
M. C.	132	66	80	98	-
S. B.	38	72	59	39	84
J. K.	19	27	53	58	60

observations indicate a fundamental importance of sulphydryl groups in radiation damage. Therefore it appears that a further study of the end-products of sulphydryl oxidation would be of value.

4.6 Cysteic Acid

As it is another product of sulphur metabolism and it is suggested that cysteic acid is the precursor of taurine (see Figure 5), it was thought to be of interest to investigate cysteic acid excretion following X-irradiation. The results are shown in Table 4.6. There is, if anything, a tendency to decreased cysteic acid output which could well be a reflection on the increased urinary taurine observed as a result of the increased decarboxylation of cysteic acid. However, as with the cystine results, (Table 4.3) the low mean output figure on day 4 post-irradiation may be artificial as a result of paucity of statistical evidence. Also, an increased excretion of cysteic acid in rats following exposure to X-irradiation has been reported (Kay, Harris and Entenman, 1956). The observations here would not appear to support this, and there is apparently little significant response at the low levels of exposure in these cases.

4.7 BAIBA

A non-protein amino acid, it is one of the few

Table 4.7 Urinary β-Amino Isobutyric Acid (μM/24 hr)

Subject	PRE	Day 1	Day 2	Day 3	Day 4
Testicular Tumour					
J. McC.	76	117	146	175	-
R. W. C.	172	82	55	111	134
R. S.*	2290	2470	940	1660	2160
J. T. M.	204	128	260	53	-
R. S. C.	140	207	193	178	-
J. R. N.*	576	866	595	394	-
J. S.	129	50	205	143	59
A. M.	175	318	152	168	-
J. R. B.	310	327	406	286	199
A. B.	67	93	117	56	26
N. B. B.	47	100	67	50	73
J. D. M.	120	166	213	187	120
D. C. R.	72	87	269	206	350
A. J.	82	168	219	256	189
Ankylosing Spondylitics					
T. C. B.	41	115	-	-	-
W. T.	114	125	414	166	-
W. B.	122	190	257	353	-
M. M.*	3910	4000	3110	2850	1976
M. C.	174	188	222	178	-
S. B.	120	234	168	113	219
J. K.	15	103	133	149	201

*High Excretor

Table 4.8 Changes in Urinary Excretion of BAIBA in Human after Irradiation

Patient	Condition	Maximum Rise Above Pre-Irradiation Level (μM/24 hr)	Day of Maximum Rise After Irradiation
J. Mc.C.	Seminoma	99	+ 3
R.W.C.	"	-	-
R.S.*	"	180	+ 1
J.T.M.	"	56	+ 2
R.S.C.	"	67	+ 1
J.R.N.*	"	290	+ 1
J.S.	"	76	+ 2
A.M.	"	143	+ 1
J.R.B.	"	96	+ 2
A.B.	"	50	+ 2
N.B.B.	"	53	+ 1
J.D.M.	"	93	+ 2
D.C.R.	"	197	+ 2
A.J.	"	174	+ 3
T.C.B.	Spondylitis	74	+ 1
W.T.	"	300	+ 2
W.B.	"	231	+ 3
M.M.*	"	90	+ 1
M.C.	"	48	+ 2
S.B.	"	114	+ 1
J.K.	"	184	+ 4

*An Excretor.

All except R.W.C. showed a rise in urinary BAIBA at some point following X-irradiation.

naturally occurring amino acids with a β-configuration. It was first isolated from human urine by Crumpler, Dent, Harris and Westall (1951) and Fink, Henderson and Fink (1951), and it has been shown (Fink et al, 1956) that BAIBA could be formed from thymine in the rat although other metabolic routes were considered possible. Awapara and Shullenberger (1957) in their work on the effect of thymine and nitrogen mustard on urinary BAIBA excretion have shown that man also converts thymine to BAIBA, and in the human being, reduction of thymine to BAIBA appears to be the major reaction (Gartler, 1959). Coon and his associates (1955, 1957), working with the pig, have indicated that valine may be another source of BAIBA.

Increased urinary BAIBA following X-irradiation has been reported (Rubini, Cronkite, Bond and Fliedner, 1959; Gerber, Gerber, Kurchara, Altman and Hempelman, 1961; Phipps and Kretchmar, 1960; Meichen and Short, 1963). A survey of Tables 4.7 and 4.8 indicates significantly elevated levels of BAIBA in the urine after X-irradiation, even on the fourth day after exposure. (c.f. Taurine). Like taurine, there would appear to be a definite response to low level exposure. The pathway of BAIBA metabolism (Figure 6) which has been elucidated largely by Fink and her associates indicates that excessive

METABOLIC REACTIONS CONCERNED WITH THE FORMATION AND DEGRADATION OF BAIBA

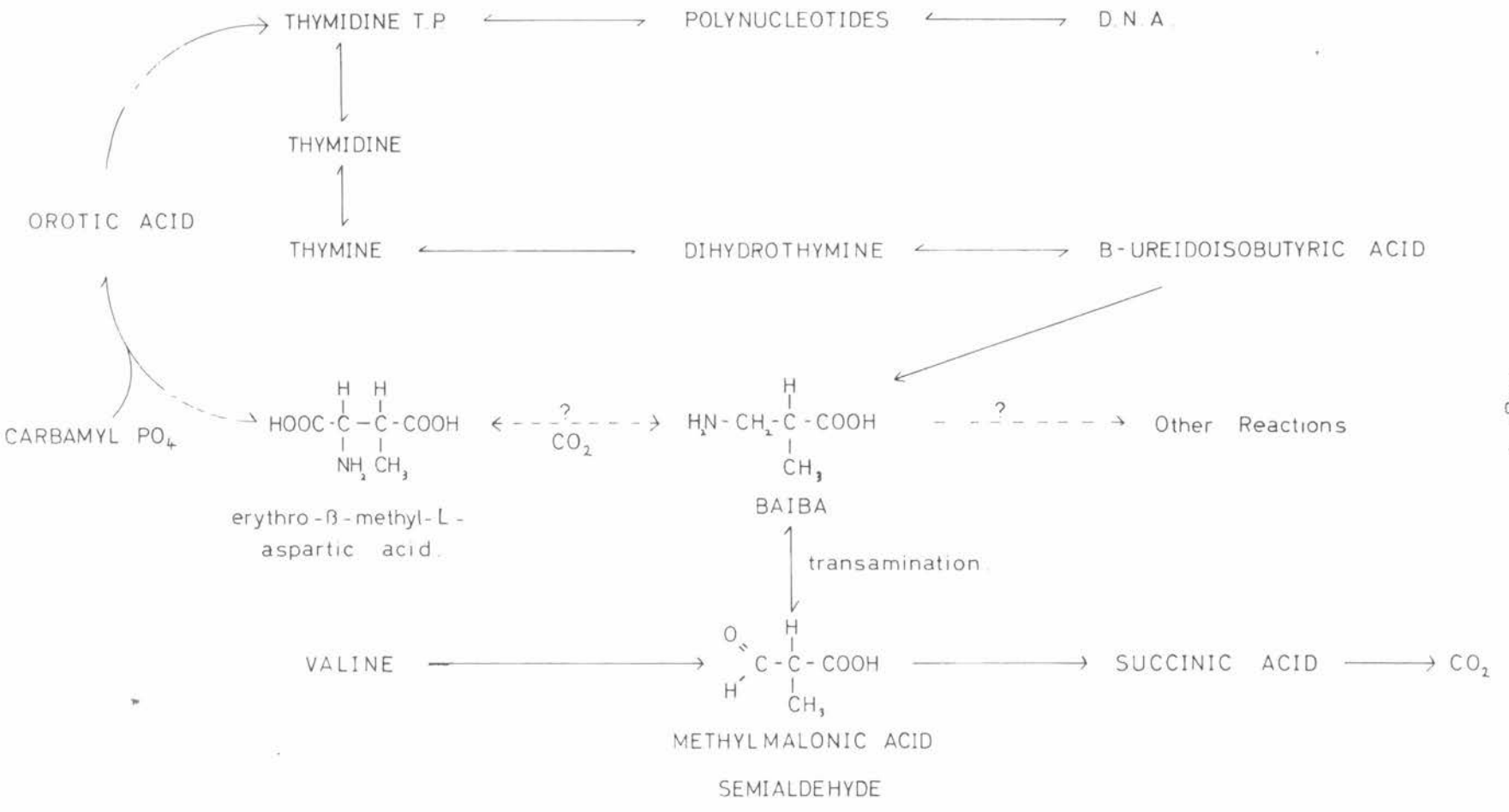
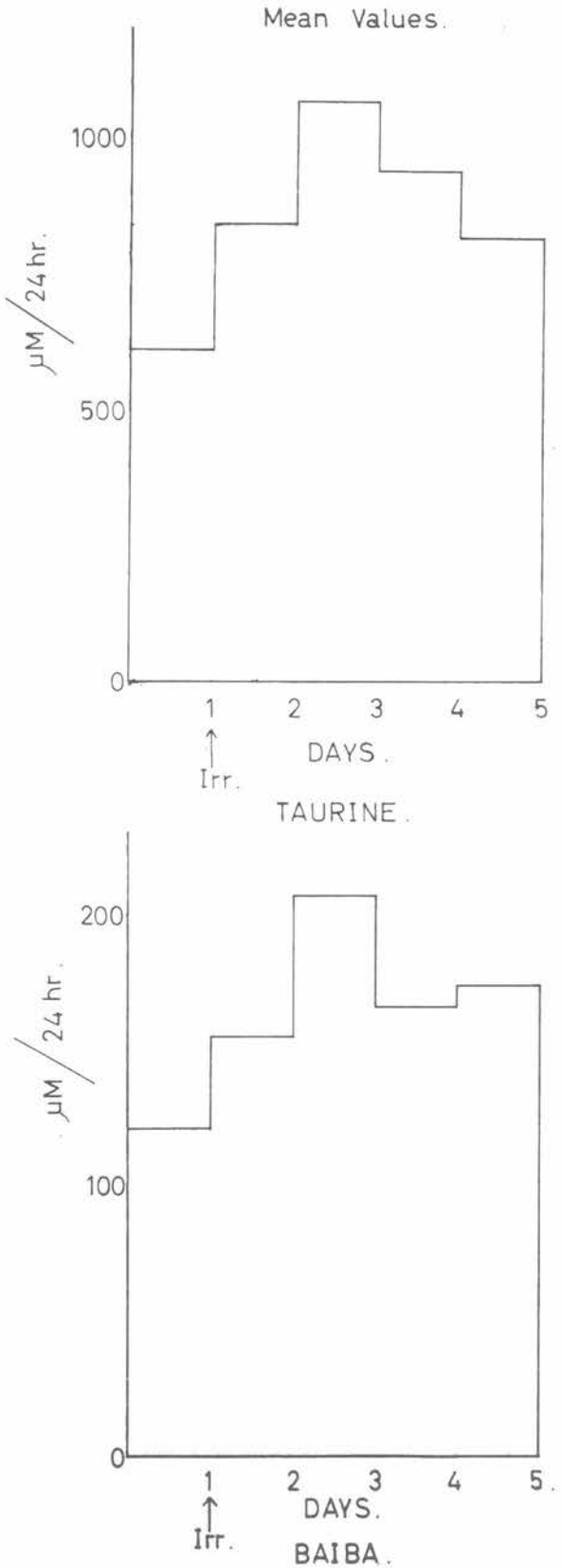


Fig. 6

Fig. 7

URINARY TAURINE AND BAIBA.



urinary BAIBA might be as a result of the increased breakdown of the genetically important DNA.

Although the changes observed in BAIBA output in this series of studies are perhaps not very great, observations at higher levels of exposure leave little doubt that there is a marked increase in the urinary output of this metabolite (Table 6.5a) and as a result of its suggested relationship to DNA catabolism, it is probable that it is a metabolite well worthy of further investigation as an indicator of exposure to radiation.

4.8 Summary of Preliminary Survey

The results of these preliminary investigations were rather disappointing. With the exception of two of the metabolite studied, namely taurine and BAIBA (Figure 7), little response to X-irradiation was observed, indicating that the levels of exposure to which the patients were subjected, were perhaps too low to be of major biochemical significance. This is a limitation which must be accepted, in that it was of necessity impossible to interfere with the normal clinical treatment. The other four metabolites did not alter outwith the normal daily ranges, and it was obviously of little value to continue a general survey of the amino acid pattern following these low levels of irradiation.

Table 4.9 Summary of Changes in Urinary Amino Acids (μM/24 hr)

AMINO ACID	Day	Low	High	Mean	Mean % Change	Number of Observations
SERINE						
	PRE	145	1140	550	-	28
Range (from literature)	1	121	1295	498	- 10.5%	28
257 - 620 Mean 400	2	263	1205	602	+ 9.5%	27
Observed 145 - 1140 Mean 550	3	170	1320	629	+ 14.5%	25
	4	160	880	502	- 9.5%	11
ETHANOLAMINE						
	PRE	49	860	375	-	27
Range 77.6- 375 Mean 200	1	135	990	469	+ 25 %	27
	2	187	2930	504	+ 34.5%	26
Observed 49 - 860 Mean 375	3	164	669	379	+ 1 %	23
	4	60	507	290	- 29 %	12
CYSTINE						
	PRE	1	142	53	-	28
Range 12.5- 138 Mean 58	1	12	162	59	+ 11 %	28
	2	8	176	67	+ 26 %	27
Observed 1 - 142 Mean 53	3	6	201	55	+ 4 %	25
	4	5	103	38	- 40 %	12
TAURINE						
	PRE	97	1320	612	-	24
Range 350 - 1850 Mean 985	1	207	2600	842	+ 38 %	24
	2	319	3740	1066	+ 75 %	23
Observed 97 - 1320 Mean 612	3	234	2720	939	+ 54 %	21
	4	258	1367	817	+ 34 %	12
CYSTEIC ACID						
	PRE	19	170	97	-	28
	1	27	216	96	- 1.5%	28
	2	22	180	99	+ 2 %	27
Observed 19 - 170 Mean 97	3	25	147	85	- 14 %	25
	4	32	123	69	- 41 %	12
β-AMINO ISOBUTYRIC ACID						
	PRE	15	310	121*	-	18*
Range 58 - 360 Mean 210	1	50	327	155*	+ 28 %	18*
	2	55	414	207*	+ 71 %	17*
Observed 15 - 310* Mean 121*	3	50	353	166*	+ 37 %	17*
	4	26	350	174*	+ 44 %	9*

*Excluding 3 High Excretors

It has been maintained that biochemical reactions in cells and tissues are but little influenced by ionising radiation. Thus it is suggested that the doses needed to produce changes in the normal concentrations of cellular components or in the rate of biochemical processes are usually far above those that cause structural lesions or even lethal effects, and there are reasons to believe that biochemical effects are mostly secondary consequences of gross lesions. However, there are possible exceptions, and two of these are:-

(A) Sulphydryl oxidation and related sulphur metabolism.

(B) Synthesis of DNA and cell division.

If it is true that certain molecules are far more radio-sensitive than others, then the two aforementioned possibilities might well account for increased taurine and BAIBA respectively in man after exposure to low doses of ionising radiation, in the absence of a general amino-aciduria. The results that have been obtained tend to indicate that these two metabolites are perhaps more radio-sensitive than the other amino acids and related compounds. This finding is in agreement with Bigwood and Soupart's observations (1962), which, at low levels of exposure, indicated a 10-fold increase in urinary

taurine and a 4-fold increase in urinary BAIBA in the absence of a general amino aciduria.

It is perhaps interesting to note that if, as has been suggested, cystinuria is considered to be the most sensitive index of liver damage and impaired amino acid metabolism (Walshe, 1953), then it would appear from these observations on cystine that the liver, which is considered to be the major site of amino acid metabolism, is presumably not a particularly radio-sensitive site, because there is no difference in response between abdominally and spinally irradiated patients. It is possibly therefore not the site of the lesion which results in the increased amounts of taurine and BAIBA in the urine after irradiation.

5. TAURINE

5.1 Response to Ionising Radiation

The sulphur atom has long been recognised as occupying a key position in both radiation injury and chemical protection against this injury. In 1954, Kay and Entenman reported that taurine (2-aminoethane sulphinic acid) was elevated in the urine of rats after exposure to whole-body X-irradiation. The original observation has been confirmed by other investigators, including Mefferd and Martens (1955), Abei et al (1957) and Pentz (1958). Kay and Entenman (1958) have also studied certain forms of partial body X-irradiation in order to determine the site responsible for the elevation of urinary taurine after irradiation. Several investigations appear to have been made in man (Hempelman et al, 1952; Andrews et al, 1959; Bigwood and Soupart, 1962; Katz and Hesterlik, 1955) and in dogs (Kossalka et al, 1955).

Thus it is well established that in several species, exposure to penetrating radiations give rise to an increased excretion of urinary taurine. However, results from a study by Angel and Noonan (1961) indicate that the excretion of taurine is species-dependant. Therefore if taurine is to be considered as a possible candidate for a bio-chemical indicator of irradiation damage, this is a

factor which must be taken into account before extrapolating animal results to human studies. For example it is generally agreed by most authors that the occurrence of the maximum peak of taurine excretion in the rat is to be observed during the first 24 hours. In these studies on man, the mean maximum of taurine excretion appears to occur on the second day post-irradiation (Table 4.4). However a study of the individual responses (Table 4.5) would appear to indicate the peak of excretion is between 18-36 hours post-irradiation with possibly a secondary peak of excretion occurring about day 4. This is possibly in agreement with the secondary wave of amino aciduria as reported by other authors (Ganis, Hendrickson and Howland, 1965).

In the cases sited by Hempelman et al (1952) on the day after exposure there was always an increased excretion of taurine even in the absence of increases in urinary amino acids. This was reported true for estimated doses as low as 35 rad. In the majority of cases studied in this first series, there was an increase in urinary taurine at some time after exposure in agreement with Hempelman's observations (Table 4.5) but in view of the dependancy of urinary taurine on the diet, (White, 1935), (Portman and Mann, 1956), it is quite probable that 35 rad

would be too low an exposure to produce a significant increase in the excretion of this metabolite in man outwith the normal daily range.

5.2 Possible causes of observed increase in Urinary Taurine

In the rat the rather large increases in taurine excretion after irradiation do not appear to be due to an increase in glomerular filtration rate since creatinine excretion, which is considered to be an index of filtration rate (Friedman, 1947) is not appreciably increased. Observations in this laboratory, applied to human studies, indicate that creatinine excretion in man also, is not appreciably altered, at low levels of exposure, which probably precludes increased glomerular filtration in man.

There are several other possibilities however, for the source of increased taurine in the urine following exposure to X-irradiation, among which are:-

(i) Oxidation of sulphur-containing compounds to taurine.

(ii) Release of pre-formed taurine from a tissue damaged by exposure to X-irradiation.

(iii) Interference in conjugation of taurine with cholic acid.

(iv) Decreased reabsorption of taurine by the kidney.

5.3 Oxidation of sulphur-containing compounds of Taurine

It was suggested that the increase in excretion of taurine arises from increased oxidation of sulphydryl compounds (Kay and Entenman, 1959; Pentz, 1958; Stern and Stim, 1959) since these substances were thought to be unusually radio-sensitive, and cystine and glutathione may be precursors of taurine. However, early work on the in vitro radiation sensitivity of sulphydryl-dependant enzymes has been questioned (Phil, Lange and Eldjarn, 1958); sulphydryl enzyme-systems display no special sensitivity in vivo, and there is no general decrease in the sulphydryl content of tissues from irradiated animals (Bacq and Alexander 1955). Ashwood-Smith (1961) found an early loss of glutathione from the thymus, but, as he pointed out, this may be no more than an expression of cell-death. Whatever the sensitivity of sulphydryl compounds, it is difficult to conceive a radiochemical mechanism which would allow production of the reported substantial amounts of taurine actually excreted after moderate radiation exposures. In particular, it surely does not explain the failure of doses above 250 rad to further increase taurine excretion (Kay, Early and Entenman, 1957).

5.4 Liberation of pre-formed Taurine

The second hypothesis is that the taurine is liberated

from a source already present in the tissues. Striated muscle has a high concentration of taurine, but there is no morphological evidence of radiation damage to muscle at the effective exposure levels. On the other hand, lymphoid tissue not only contains substantial concentrations of taurine in rats and mice (Kit and Awapara, 1953), but is readily injuried by radiation, apparently in a time-sequence which matches the observed excretion of taurine (Watson, 1962). The obvious conclusion from this, is that perhaps radiation damage to lymphoid tissues liberates the taurine found in the urine after exposure.

It is a fact that atrophy of lymphoid tissues does occur after irradiation, demonstrable by a lymphopenia and histological changes in biopsy material. The fact that a hypertaurinuria occurs in the absence of a general aminoaciduria would support the hypothesis, but it does seem an anomaly that the spleen is not involved in the rat, yet this organ is rich in taurine (Kay, 1959). In addition, if the excess excretion of taurine is due to liberation of pre-formed taurine, it is again surprising that the taurine excretion does not increase as the dose of irradiation is increased above 250 rad. (Kay, 1957).

5.5 Possible Renal Effect

A third hypothesis is that increased taurine

excretion is a result of decreased reabsorption of taurine by the kidney. It has been reported (Gilbert et al, 1959) that intraperitoneal administration of amino acids to the mouse causes an increase in urinary taurine. In particular, the β-amino acids were found to produce changes in taurine concentration greater than those observed with most of the other amino acids. One explanation for these observations is that certain administered amino acids have the capacity to compete with taurine for cell membrane transport in the kidney tubule. Similar effects have been reported in humans (Robson and Rose, 1957; Derrick and Hanley, 1957). Therefore it is possible that taurine is displaced from its site of reabsorption in the kidney by certain amino acids previously administered. And certain β-amino acids, namely β-alanine, BAIBA and β-amino-n-butyric acid, appear to compete specifically with taurine for tubular reabsorption (Gilbert, 1959).

Therefore the fact that hypertaurinuria is an early and positive result of radiation in the absence of a general amino aciduria, might be explained by the fact that the amino acids are preferentially reabsorbed in the kidney tubules at the expense of the taurine in the glomerular filtrate.

As a working hypothesis the hypertaurinuria following exposure to X-irradiation might be explained as a result of a combination of two factors. First at low levels of X-irradiation, taurine and amino acids are released from the disintegrating lymphocytes, but in the kidney tubules the amino acids competitively inhibit taurine reabsorption, resulting in relatively high urinary taurine excretion. At higher levels of exposure, it is reasonable to assume that more amino acids are released and under these circumstances, saturation of the renal tubular mechanism occurs, resulting in a general amino aciduria. For example, exposure of a monkey to 900 rad total body X-irradiation produced such a picture (Table 6.5a).

5.6 Summary

To sum up the study of taurine. It seems indicative that there is a significant rise in taurine in the urine of man following low doses of partial-body X-irradiation and it may be possible that in the absence of a general amino-aciduria, this is an early and specific manifestation of radiation damage and therefore of diagnostic value. But there are a number of objections to its adoption as a bio-chemical indicator:-

(i) Taurine excretion is somewhat variable

amongst normal individuals and is very much influenced by dietary intake. It reflects primarily protein intake as evidenced by its lowered excretion under fasting conditions. These are serious limitations if one desires to use the level of taurine excretion as an indicator of the amount of ionising irradiation to which an individual has been exposed. It should be borne in mind however, that perhaps these conditions could be controlled in studies involving hospitalised patients.

(ii) The observations of Kay and Entenman (1957) and Angel and Noonan (1961), would appear to indicate that increased taurine excretion following exposure to X-irradiation is species dependant.

(iii) It has been reported (Hempelman et al, 1952) that on the day after exposure there was always increased excretion of taurine in rats, even in the absence of increases in urinary amino acids, and further that this was true for doses as low as 35 rad. This would appear to indicate that hypertaurinuria is a radio-sensitive response, but it is doubtful if this reaction

to such low doses would be readily observed in man. The results from this series tend to indicate only a trend to a definite response at 150-300 rad partial-body, which is approximately equivalent to 35 rad whole-body exposure on a crude extrapolation basis. Also, it has been reported (Kay et al, 1957) that in rats, up to 250 rad exposure, the excretion of taurine rises sharply, but thereafter no further increases in taurine excretion occur on increasing the dose. If such a response is also to be found in man, this effect would also limit the use of taurine as a biochemical indicator.

One further point. Gilbert and his co-workers (1960) reported that the β-amino acids when injected intraperitoneally into the mouse produced high urinary concentrations of taurine, and increased the total urinary excretion of taurine. They concluded that the high concentrations of administered β-amino acids competitively displaced taurine from its usual reabsorption sites in the renal tubule. Might it not be, therefore, that it is in fact BAIBA which is the major radio-sensitive metabolite, and as a

Table 5.1 Comparison of Excretion Patterns of Taurine and β-Aminoisobutyric Acid Mean Values (μM/24 hr)

Amino Acid	Pre	Day 1	Day 2	Day 3	Day 4
Taurine	612	842	1066	939	817
BAIBA	121	155	207	166	174
Ratio Taurine/ BAIBA	5.06	5.43	5.16	5.62	4.70

*excluding 3 high excretors

result of its excessive formation from DNA disintegration and subsequent competitive displacement of taurine in the kidney tubules, it produces, or at least contirbutes to, the hypertaurinuria observed after exposure to low-level X-irradiation. A study of Tables 4.5, 4.8 and 4.9) indicates that the mean maximum of both taurine and BAIBA excretion in man appears to occur on the second day post-exposure, although there was admittedly a fairly wide individual variation. Table 5.1 shows a comparison of the excretion patterns of taurine and BAIBA. The ratio taurine/BAIBA would appear to remain remarkedly constant. Might this not be indicative of some metabolic relationship between taurine and BAIBA, at any rate, as regards reabsorption in the kidney tubules.

To conclude taurine excretion, although further study would appear to be of value, it was decided to discontinue taurine studies for the time being, bearing in mind the serious limitations with a view to use as a biochemical indicator imposed by dependancy on diet and species, and to concentrate upon pyrimidine metabolism, in particular the metabolism of thymine and BAIBA.

TABLE 5.2 Plasma Serine (μM/100 ml)

Subject	C	C + 3 hr.	C + 24	X + 3	X + 12	X + 24	X + 27	X + 48	X + 51	X + 72
D.W.	19.7	15.9	26.1	19.4	24.6	70.5	79.5	14.3	18.1	-
T.G.W.	11.5	11.3	-	10.5	12.3	13.9	9.3	12.6	11.4	22.3
O.M.H.	16.6	19.6	-	4.9	15.6	11.1	10.9	17.0	18.7	7.4
G.S.H.	26.3	27.8	22.3	19.8	-	20.0	20.6	24.1	-	-
D.Mc.K.	22.6	30.9	-	48.5	28.6	23.7	28.1	26.0	-	-
I.Mc.D.	34.4	42.4	-	29.0	-	32.3	33.3	22.6	37.9	42.0
E.D.C.	17.2	17.8	16.4	14.3	13.2	18.8	17.4	17.3	18.9	20.2
Mean	21.2	23.7	21.3	21.2	18.9	27.2	28.4	19.1	21.0	23.0
High	34.4	42.4	26.1	48.5	28.6	70.5	79.5	26.0	37.9	42.0
Low	11.5	11.3	16.4	4.9	12.3	11.1	9.3	12.6	11.4	7.4
No. of Obs.	7	7	3	7	5	7	7	7	5	4

C - Control

X - Irradiated

Table 5.3 Plasma Ethanolamine (μM/100 ml)

Subject	C	C + 3hr.	C + 24	X + 3	X + 12	X + 24	X + 27	X + 48	X + 51	X + 72
D.W.	0.10	0.25	0.55	0.80	0.80	0.57	0.75	0.80	0.47	-
T.G.W.	0.32	0.17	-	0.73	0.57	0.85	0.47	0.44	0.83	1.29
O.M.H.	2.08	0.50	-	1.27	3.65	3.77	2.55	3.74	2.94	1.86
G.S.H.	0.36	0.64	0.95	0.75	-	1.01	0.74	0.53	-	-
D.Mc.K.	1.15	2.81	-	0.77	0.76	1.07	1.12	0.71	-	-
I.Mc.D.	2.64	3.01	-	2.34	-	2.28	1.39	2.07	2.09	1.32
E.D.C.	1.03	1.31	1.29	1.20	0.87	0.45	0.79	0.67	0.96	1.02
Mean	1.10	1.24	0.93	1.12	1.33	1.43	1.12	1.28	1.46	1.37
High	2.64	3.01	1.29	2.34	3.65	3.77	2.55	3.74	2.94	1.86
Low	0.10	0.17	0.55	0.73	0.57	0.45	0.47	0.44	0.47	1.02
No. of Obs.	7	7	3	7	5	7	7	7	5	4

C - Control

X - Irradiated

Table 5.4 Plasma Cystine (μM/100 ml)

Subject	C	C + 3hr.	C + 24	X + 3	X + 12	X + 24	X + 27	X + 48	X + 51	X + 72
D.W.	3.92	3.03	2.71	2.70	2.66	3.19	3.56	2.24	2.61	-
T.G.W.	2.40	2.00	-	2.70	2.50	2.09	2.21	2.89	2.74	2.55
O.M.H.	2.04	2.32	-	1.21	3.65	3.77	2.55	3.74	2.94	1.86
G.S.H.	4.34	3.04	3.48	3.69	-	3.89	3.38	3.97	-	-
D.Mc.K.	2.48	3.86	-	2.03	2.85	2.29	2.65	2.75	-	-
I.Mc.D.	3.81	4.55	-	2.95	-	3.74	3.69	4.75	4.25	3.16
E.D.C.	3.80	3.76	3.15	2.59	2.61	3.46	3.66	3.07	3.35	3.52
Mean	3.26	3.22	3.11	2.55	2.85	3.23	3.10	3.34	3.18	2.77
High	4.34	4.55	3.48	3.69	3.65	3.89	3.69	4.75	4.25	3.52
Low	2.04	2.00	2.71	1.21	2.50	2.09	2.21	2.24	2.61	1.86
No. of Obs.	7	7	3	7	5	7	7	7	5	4

C - Control

X - Irradiated

Table 5.5 Plasma Taurine (μM/100 ml)

Subject	C	C + 3hr.	C + 24	X + 3	X + 12	X + 24	X + 27	X + 48	X + 51	X + 72
D.W.	3.76	3.19	3.65	5.82	6.57	3.07	2.80	3.22	7.34	-
T.G.W.	2.88	2.11	-	2.11	1.92	1.81	1.65	1.96	2.67	3.82
O.M.H.	4.82	3.77	-	3.55	7.83	4.98	2.33	3.94	2.84	2.83
G.S.H.	3.57	4.01	2.81	4.60	-	2.00	3.85	3.46	-	-
D. Mc.K.	5.10	9.62	-	4.03	8.35	4.50	6.95	3.70	-	-
I.Mc.D.	8.05	10.70	-	9.40	-	6.80	5.50	10.40	12.40	6.40
E.D.C.	4.72	5.30	4.24	3.21	4.54	3.65	3.16	3.73	9.77	3.29
Mean	4.70	5.53	3.57	4.67	5.96	3.83	3.75	4.34	7.00	4.08
High	8.05	10.70	4.24	9.40	8.35	6.80	6.95	10.40	12.40	6.40
Low	2.88	2.11	2.81	2.11	1.92	1.81	1.65	1.96	2.67	2.83
No. of Obs.	7	7	3	7	5	7	7	7	5	4

C - Control

X - Irradiated

Table 5.6 Plasma Cysteic Acid (μM/100 ml)

Subject	C	C + 3 hr.	C + 24	X + 3	X + 12	X + 24	X + 27	X + 48	X + 51	X + 72
D.W.	0.22	0.16	0.34	0.62	0.45	0.49	0.52	0.48	0.34	-
T.G.W.	0.48	0.37	-	0.21	0.16	0.16	0.22	0.38	0.39	0.39
O.M.H.	0.69	0.48	-	0.34	0.59	0.40	0.32	0.52	0.65	0.63
G.S.H.	0.31	0.30	0.52	0.58	-	0.69	0.44	0.30	-	-
D.Mc.K.	0.59	0.53	-	0.36	0.40	0.38	0.49	0.50	-	-
I.McD.	0.54	0.85	-	0.82	-	0.68	0.74	0.81	0.50	0.53
E.D.C.	0.38	0.36	0.41	0.37	0.40	0.42	0.37	0.38	0.58	0.49
Mean	0.46	0.44	0.42	0.47	0.40	0.46	0.44	0.48	0.49	0.51
High	0.69	0.85	0.52	0.82	0.59	0.68	0.74	0.81	0.65	0.63
Low	0.22	0.16	0.34	0.21	0.16	0.16	0.22	0.30	0.34	0.39
No. of Obs.	7	7	3	7	5	7	7	7	5	4

C - Control

X - Irradiated

5.7 Changes in Plasma Amino Acids

Before proceeding to a discussion on BAIBA metabolism, it was considered appropriate to report at this stage that during the course of BAIBA studies, a few observations were reported on the plasma levels of serine, ethanolamine, cystine, taurine and cysteic acid. These results are shown in Tables (5.2-5.6). As expected, with the urine studies in mind, these results were also rather disappointing, indicating once again that the levels of X-irradiation were too low to promote any major alteration in the plasma metabolites followed.

With the exception of one case D.W. who showed a remarkable increase in plasma serine 24 hours post-irradiation (in view of the other results the possibility of experimental error cannot be excluded in this case), there were no significant alterations in any of these 5 plasma metabolites in any of the patients studied. In particular, unlike urinary taurine, there was little increase in the plasma taurine levels, except perhaps a slight tendency to a rise about 2 days after exposure. In view of the fairly significant hypertaurinuria observed, it seems likely that there is an important renal mechanism resulting in the elevation of taurine in the urine after exposure to X-irradiation.

6. BAIBA $H_2N.\ CH_2 - CH(CH_3) - COOH$

6.1 Introduction

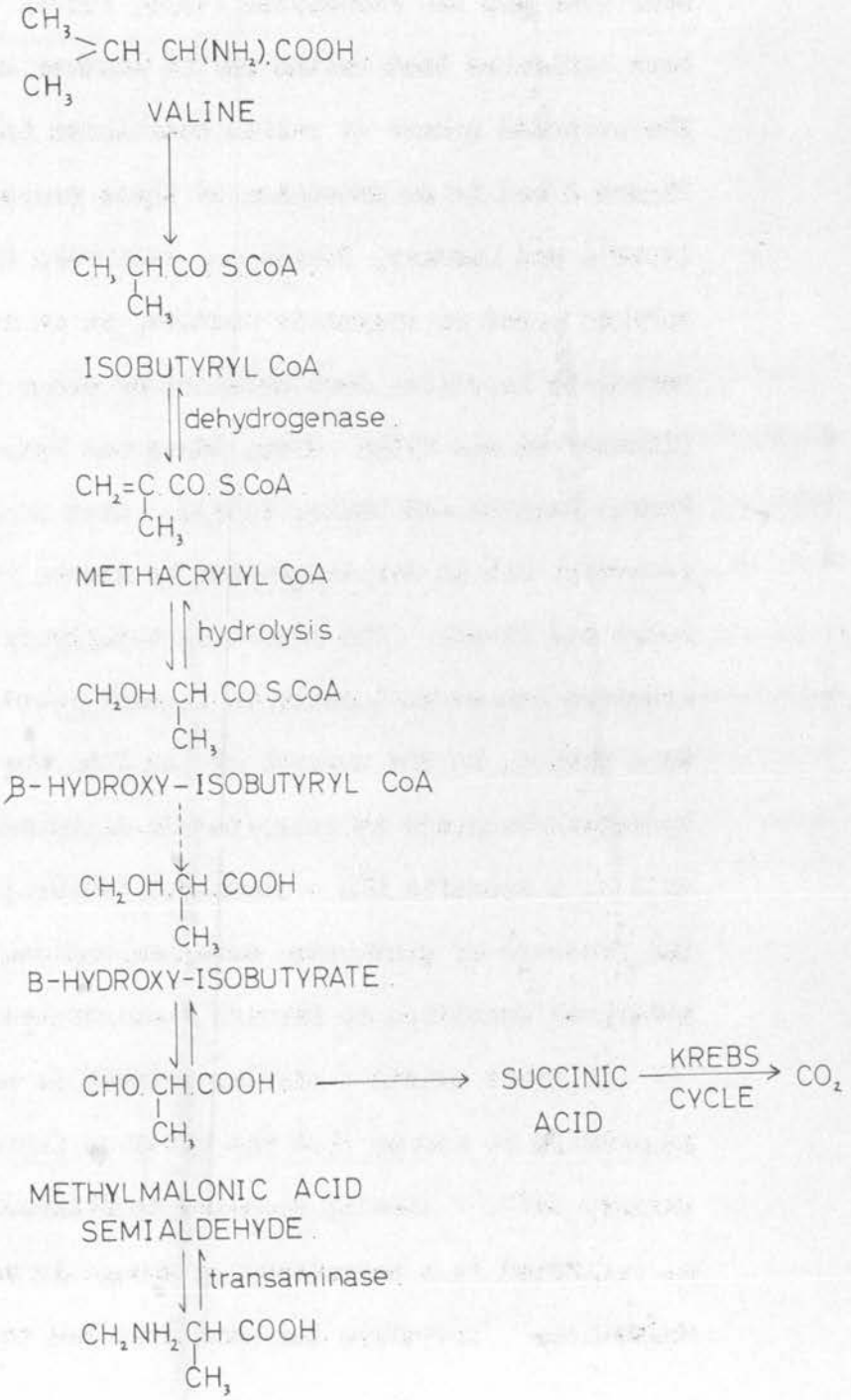
β-aminoisobutyric acid (BAIBA) is a non-protein amino acid found in human urine in widely varying concentrations from one individual to another. Several studies have demonstrated that this variation is largely under genetic control (Harris 1953; Gartler, Firschein and Kraus, 1957). However, the amount of BAIBA excreted per day is reported to be constant for a given individual, and there seems to be no obvious relationship between such excretion and diet or sex and age (except in infants under 5 years of age). That is to say, the trait is characteristic of individuals and largely independent of environmental factors (Harris 1953; Gartler 1956). Therefore the fact that the pre-irradiation levels of an individual tend to be relatively constant, makes BAIBA a very suitable metabolite for study with a view to establishing a satisfactory bio-chemical indicator of irradiation induced damage in man.

As has been previously stated, work by Fink et al., established that BAIBA is formed from pyrimidines (Fink et al. 1952a, 1952b, 1956a, 1956b). They have demonstrated this conversion from thymine in the rat (1956c).

6.2 Valine - A possible source of BAIBA -

Fig. 8

PROPOSED SCHEME OF VALINE AND BAIBA METABOLISM



In the human being, reduction of thymine to BAIBA appears to be the major reaction (Gartler, 1959). However Coon and his associates (1955, 1957a, 1957b) in pig have indicated that valine may be another source of BAIBA. The proposed scheme of valine metabolism is shown in Figure 8 and is an extension of those proposed by Atchley (1948), and Kinnory, Takeda and Greenberg (1955). This scheme, based on enzymatic studies, is in accord with carbon-14 labelling data obtained by other investigators (Kinnory et al. 1955; Gray, Adams and Hauptmann, 1950; Fones, Waalkes and White, 1951). Coon suggests that isobutryl CoA is dehydrogenated by enzyme fractions of heart and liver. The resulting methylacrylyl CoA is hydrated non-enzymatically to furnish β-hydroxyisobutryl CoA, whence, on the removal of the CoA, the free β-hydroxyisobutyrate is converted to methylmalonaldehydic acid by a specific DPN - dependent dehydrogenase. In the presence of glutamate, methylmalonaldehydic acid undergoes amination to furnish β-aminoisobutyrate.

If there exists a similar pathway in man, it is reasonable to assume that the observed increase in urinary BAIBA following exposure to X-irradiation, might be reflected in a corresponding change in valine excretion. Therefore the next step was to undertake a

Table 6.1 Urinary Valine (μM/24 hr)

Subject	PRE	Day 1	Day 2	Day 3	Day 4
Testicular Tumour					
J. McC.	66	115	36	48	-
R. W. C.	40	96	171	49	57
R. S.	44	120	25	85	27
J. T. M.	22	22	54	33	-
R. S. C.	139	169	156	138	-
J. R. N.	64	88	43	35	-
J. S.	83	180	75	142	76
A. M.	87	87	71	70	-
J. R. B.	60	70	119	52	43
A. B.	23	63	109	76	40
N. B. B.	43	51	74	43	63
J. D. M.	65	100	68	92	49
D. C. R.	56	67	83	99	85
A. J.	48	40	65	50	48
Ankylosing Spondylitics					
T. C. B.	27	55	-	-	-
W. T.	26	69	74	52	-
W. B.	42	48	63	46	-
M. M.	109	152	188	136	82
M. C.	78	44	112	84	-
S. B.	31	64	49	45	53
J. K.	38	45	65	66	64

	PRE	Day 1	Day 2	Day 3	Day 4
Number of Observations	21	21	20	20	12
Mean	57	83	85	72	57
Mean % Change	-	+ 45%	+ 49%	+ 26%	-
High	139	180	188	142	85
Low	22	22	25	33	27

Table 6.2 Urinary Glutamic Acid (μM/24 hr)

Subject	PRE	Day 1	Day 2	Day 3	Day 4
Testicular Tumour					
J. McC.	80	69	125	200	-
R. W. C.	58	37	50	83	65
R. S.	123	147	140	68	200
J. T. M.	57	55	13	26	-
R. S. C.	83	40	62	70	-
J. R. N.	94	46	23	59	-
J. S.	66	27	22	79	36
A. M.	69	74	73	127	-
J. R. B.	86	58	46	43	76
A. B.	59	77	63	48	30
N. B. B.	26	60	47	33	41
J. D. M.	36	185	175	93	60
D. C. R.	62	54	64	63	99
A. J.	22	28	50	12	52
I. McD.	30	28	52	43	-
O. M. H.	70	34	62	82	-
T. G. W.	75	80	38	62	-
G. S. H.	97	97	109	-	-
D. W.	29	31	80	19	-
D. McK.	74	20	34	-	-
E. D. C.	56	108	181	115	-
Ankylosing Spondylitics					
T. C. B.	53	77	-	-	-
W. T.	49	82	84	75	-
W. B.	27	39	46	42	-
M. M.	53	35	90	114	90
M. C.	91	48	50	29	-
S. B.	13	54	38	39	29
J. K.	24	28	12	33	28

	PRE	Day 1	Day 2	Day 3	Day 4
Number of Observations	28	28	27	25	12
Mean	59	61	68	66	67
Mean % Change	-	+ 3.5%	+ 15%	+ 12%	+ 13.5%
High	123	185	181	200	200
Low	13	20	12	12	28

Table 6.3 Plasma Valine (μM/100 ml)

Subject	C	C + 3hr.	C + 24	X + 3	X + 12	X + 24	X + 27	X + 48	X + 51	X + 72
D.W.	29.6	26.3	23.6	22.3	24.7	24.3	28.2	19.5	22.0	-
T.G.W.	17.9	17.2	-	22.9	22.5	25.7	26.1	25.2	25.8	28.3
O.M.H.	27.7	26.2	-	14.7	23.4	30.2	23.3	37.9	30.1	26.2
G.S.H.	25.9	23.4	16.6	17.4	-	19.9	20.2	23.5	-	-
D. Mc.K.	23.7	31.4	-	20.7	27.7	23.2	27.4	30.8	-	-
I. Mc.D.	32.1	43.5	-	24.4	-	34.4	34.9	46.6	44.7	32.5
E.D.C.	37.3	31.4	27.3	21.3	19.6	28.6	27.0	26.8	30.1	25.7
Mean	27.7	28.5	23.5	20.5	23.6	26.6	26.7	30.0	30.5	28.2
High	32.1	43.5	27.3	24.4	27.7	34.4	34.9	46.6	44.7	32.5
Low	17.9	17.2	16.6	14.7	19.6	19.9	20.2	19.5	22.0	25.7
No. of Obs.	7	7	3	7	5	7	7	7	5	4

C - Control

X - Irradiated

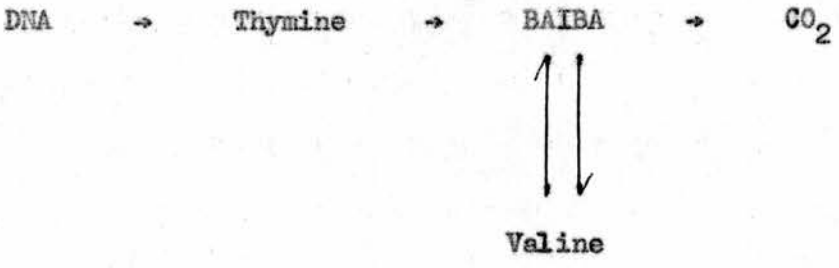
Table 6.4 Plasma Glutamic Acid (μM/100 ml)

Subject	C	C + 3 hr.	C + 24	X + 3	X + 12	X + 24	X + 27	X + 48	X + 51	X + 72
D.W.	18.9	21.1	13.9	20.8	13.9	10.9	12.2	16.0	18.7	-
T.G.W.	27.8	24.8	-	33.4	31.7	33.5	33.1	28.0	28.1	28.6
O.M.H.	25.3	31.0	-	18.9	27.2	32.7	26.0	31.7	25.3	27.3
G.S.H.	25.8	25.5	22.3	31.5	-	24.9	23.0	33.8	-	-
D.Mc.K.	31.2	44.2	-	27.4	38.1	26.6	38.0	13.9	-	-
I.Mc.D.	27.2	30.5	-	27.2	-	24.2	29.2	34.3	45.1	32.2
E.D.C.	20.0	23.7	19.7	23.0	16.0	19.5	20.0	17.4	25.7	24.2
Mean	25.2	28.7	19.0	26.0	25.4	24.6	25.9	25.0	28.6	28.1
High	31.2	44.2	22.3	33.4	38.1	33.5	38.0	34.3	45.1	32.2
Low	18.9	21.1	13.9	18.9	13.9	10.9	12.2	13.9	18.7	24.2
No. of Obs.	7	7	3	7	5	7	7	7	5	4

C - Control

X - Irradiated

study of the urinary and plasma valine levels in man following exposure to partial X-irradiation, in an attempt to establish if there was a metabolic relationship between BAIBA and valine. At the same time, it was decided to investigate the urine and plasma levels of glutamic acid, which is considered to be related to the catabolism of BAIBA. The results are shown in Tables 6.1-6.4. There were no significant changes in either of the metabolites, in both urine and plasma, but there was perhaps a slight trend to elevated urinary valine levels after exposure, returning to normal on day four. Thus, the valine pathway, if existent in man, may only be of minor significance. If the pathway postulated by Coon and his co-workers was reversible then it is feasible that a tendency to increased valine formation may result in the presence of increased BAIBA levels in the body fluids.



6.3 Animal Studies.

The next step involved a series of animal experiments to determine suitable experimental techniques for the

Table 6.5a Monkey Experiment 1 - Dose Whole Body 900 r - Urine Samples

Amino Acid	Normal Excretion Pre-Irradiation	Highest Level Excreted Post-Irrad.	Time after irrad. for highest level	Time taken from zero to return to normal
Ethanolamine	0.5 µm/hr.	2.3 µm/hr.	20 hrs.	80 hrs.
Serine	0.43 "	1.94 "	1 hr.	16 hrs.
Cystine	0.20 "	1.04 "	1 hr.	16 hrs.
Tryptophan	0.20 "	0.83 "	1 hr.	3 hrs.
Kynurenine	0.30 "	0.97 "	20 hrs.	27 hrs.
BAIBA	0.03 "	2.78 "	60 hrs.	80 hrs.
Glutamic Acid (i)	0.35 "	1.27 "	1 hr.	40 hrs.
" (ii)		1.07 "	60 hrs.	80 hrs.
β-Alanine	0.50 "	3.915 "	1 hr.	3 hrs.
Valine	0.35 "	3.57 "	1 hr.	3 hrs.
Lysine	0.50 "	6.37 "	1 hr.	3 hrs.
Tyrosine	0.18 "	3.43 "	1 hr.	16 hrs.
Phenylalanine	0.12 "	4.85 "	1 hr.	40 hrs.
Leucine	0.25 "	10.34 "	1 hr.	16 hrs.
Isoleucine	0.25 "	3.27 "	1 hr.	16 hrs.
Aspartic Acid	0.18 "	0.85 "	1 hr.	3 hrs.
Alanine	0.15 "	3.23 "	1 hr.	27 hrs.
Arginine	0.05 "	3.4 "	1 hr.	40 hrs.
Glycine	0.60 "	2.0 "	1 hr.	3 hrs.

Table 6.5b Monkey Experiment 1 - Dose Whole Body 900r - Plasma Samples

Amino Acid	Pre	Day 1	Day 2	Day 3
Ethanolamine	0.42	2.41	1.20	1.81
Serine	14.00	12.90	10.20	18.30
Cystine	1.60	3.60	1.75	2.75
Tryptophan	4.62	4.83	1.86	4.10
BAIBA	0.42	0.64	0.25	0.38
Glutamic Acid	10.30	13.80	8.35	8.10
β-alanine	0.72	0.47	0.22	0.25
Valine	17.80	44.30	22.70	29.10
Lysine	14.50	22.50	12.40	21.60
Tyrosine	3.70	5.20	3.20	4.30
Phenyalamine	3.90	7.90	3.90	5.30
Leucine	9.60	31.50	15.40	20.20
Isoleucine	5.90	17.50	8.80	11.75
Aspartic Acid	1.70	2.60	2.47	1.65
Alanine	22.20	35.0	18.40	23.10
Arginine	5.80	10.70	4.12	7.80
Glycine	27.80	48.00	23.20	33.30
Histidine	9.70	13.90	8.00	10.50
1-Methyl Histidine	2.50	4.63	5.40	4.08
Taurine	6.80	12.30	6.43	7.10

All values in μM/100 ml. Plasma

Table 6.6 BAIBA levels in the Monkey following Whole Body X-Irradiation (900 rads)

Rh Monkey 265/11

(i) Urinary BAIBA:-

Volume (ml.)	55.0	104.0	150.0	28.0	31.0	14.8	17.0	24.2	25.0	39.0	27.5	7.3
Voiding Time (hr.)	$4\frac{1}{4}$	12	$7\frac{3}{4}$	$3\frac{1}{2}$	$12\frac{1}{2}$	7	$4\frac{1}{2}$	$12\frac{1}{2}$	$11\frac{1}{2}$	$12\frac{1}{2}$	$12\frac{1}{2}$	7
Sample No.	1	2	3	4*	5	6	7	8	9	10	11	12
BAIBA (μM/sample)	0	0	0.213	2.35	6.78	5.73	3.79	7.22	25.20	34.70	6.27	1.08
BAIBA (μM/hr.)	0	0	0.03	0.672*	0.542	0.82	0.84	0.617	2.19	2.78	0.50	0.154

*900 rads whole body X-irradiation

(ii) Plasma BAIBA:- μM/100 ml. Plasma

Pre	Day 1	Day 2	Day 3
0.42	0.64	0.25	0.38

Rh Monkey 565-8

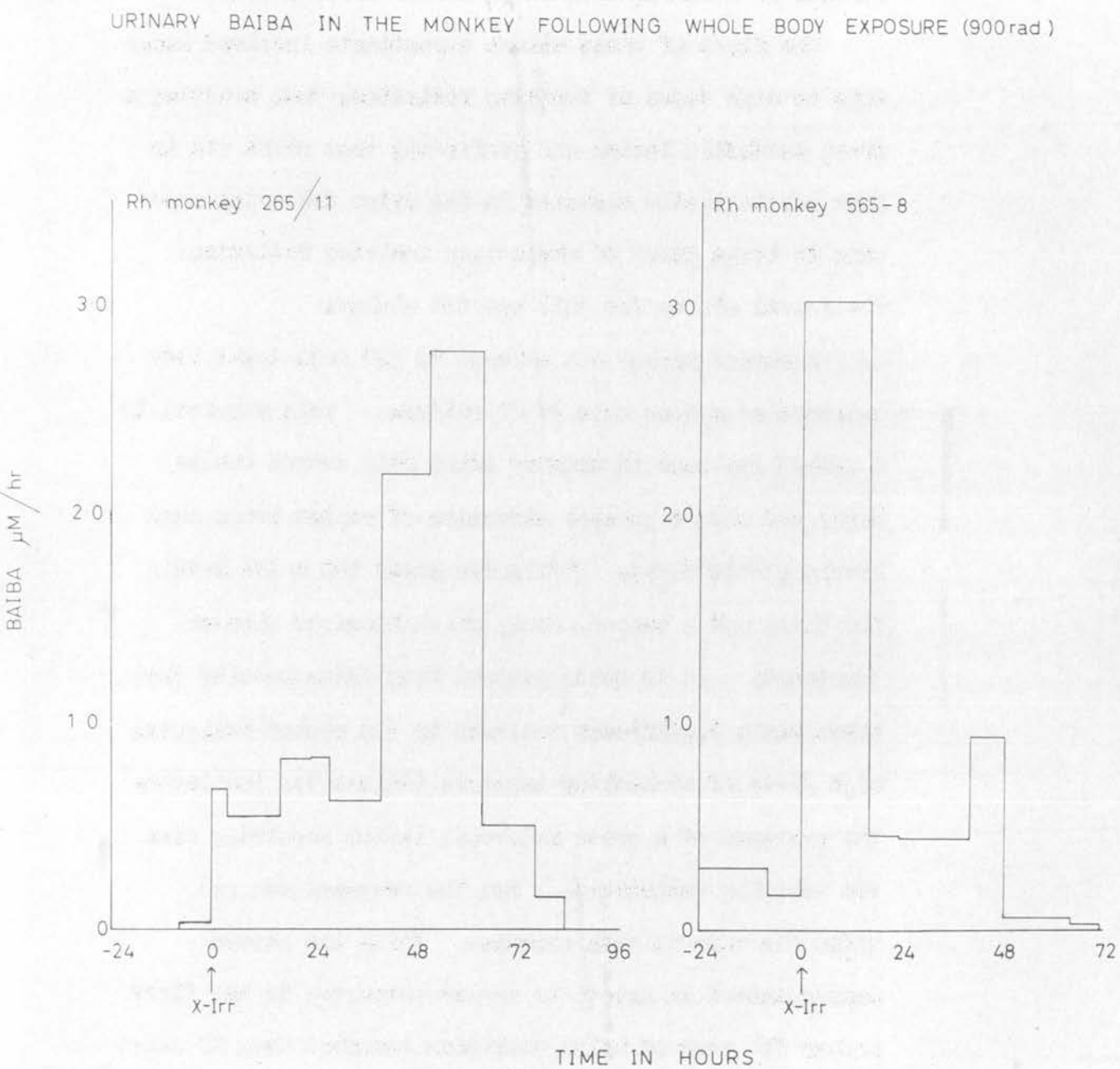
(i) Urinary BAIBA:-

Volume (ml.)	110	160	90	21	60	15	20	18
Voiding Time (hr.)	16	$8\frac{1}{2}$	16	7	17	8	16	7
Sample No.	1	2	3	4	5	6	7	8
BAIBA (μM/sample)	4.77	1.40	48.40	3.18	7.49	7.50	0.91	0.20
BAIBA (μM/hr.)	0.298	0.165	3.03*	0.454	0.441	0.937	0.06	0.03

*900 rads whole body X-irradiation

No plasma samples obtained from this monkey.

Fig. 9


URINARY BAIBA IN THE MONKEY FOLLOWING WHOLE BODY EXPOSURE (900rad)
Rh monkey 265/11
Rh monkey 565-8
BAIBA μM/hr
30
20
10
0
-24
0
24
48
72
96
X-Irr
TIME IN HOURS

study of both BAIBA metabolism in the human and the effects of X-irradiation on this metabolic system.

The first of these animal experiments involved exposure to high doses of ionising radiation, thus creating a gross metabolic lesion and confirming that BAIBA was in fact substantially elevated in the urine following exposure to large doses of whole-body ionising radiation. The animal chosen for this was the monkey.

A rhesus monkey was exposed to 900 rads total body exposure at a dose rate of 80 rad/min. This resulted in a marked increase in urinary amino acid output (Table 6.5a) and also a general elevation of plasma amino acid levels (Table 6.5b). Table 6.6 shows the BAIBA levels for this, and a second monkey which received similar treatment. It is quite evident from these results that there was a significant increase in the monkey following high doses of whole-body exposure (Figure 9), indicating the presence of a gross metabolic lesion resulting from the ionising radiations. But the response was not quite the same in both animals. While the second monkey showed an immediate marked response, in the first monkey the peak of BAIBA excretion occurred some 60 hours after exposure. This result is difficult to explain but is perhaps in agreement with the somewhat different

Table 6.7 Changes in Plasma Amino Acids in Rat after Irradiation

Amino Acid	Control	Exteriorised Intestine Irradiated (1000 r)	Whole Body Irradiated (500 r)	Whole Body + Nephrectomy Irradiated (500 r)
Cystic Acid	0.006	0.011	0.012	0.036
Taurine	0.402	1.260	0.484	>1.260
Aspartic Acid	0.050	0.075	0.093	0.102
Serine	0.259	0.262	0.290	0.298
Glutamic Acid	0.302	0.324	0.357	0.280
Citrulline	0.107	0.103	0.129	0.247
Valine	0.163	0.247	0.249	0.328
Cystine	0.050	0.033	0.050	0.032
BAIBA	0.0032	0.0036	0.0055	0.018
Ethanolamine	0.007	0.038	0.016	0.010
Tryptophane	0.080	0.060	0.060	0.044

All figures expressed in μM/ml Plasma.

Samples collected 7 hours after X-irradiation.

responses amongst individuals, observed in the human studies.

A second animal experiment was performed in an attempt to determine the influence of abdominal irradiation upon BAIBA excretion, in view of the fact that the subjects studied were subjected to therapeutic doses in the abdominal region. Twenty mature female sprague-dawley rats were divided into four groups and anaesthetised with Nembutal (3 mg/100 gram body weight i.p.). One group served as a control; the second group were destined to receive 500 rad X-rays to total body; the third group were treated in an identical manner to the total-body-irradiated animals except that a bilateral nephrectomy was performed one hour prior to irradiation; the fourth group received 1000 rad to exteriorised intestine with adequate shielding of the rest of the abdomen and body. Anaesthesia was maintained with ether during surgical manoeuvres. Samples of plasma were collected from the tail vein at 1, 4 and 7 hrs. after irradiation and the changes in amino acid concentration are shown for the 7 hr. samples (Table 6.7). It is apparent in regard to BAIBA that excessive amounts are liberated into plasma after total body irradiation and that the renal route of excretion is a major homeostatic mechanism.

It is generally accepted that the failure of plasma BAIBA concentration to increase when there is excessive BAIBA production is because of the low renal threshold for BAIBA (Armstrong et al 1963). This is in agreement with the fact that a high plasma BAIBA concentration was demonstrable after bilateral nephrectomy following irradiation, emphasising the importance of the kidneys in removing excessive amounts of BAIBA from the plasma (Smith, 1965). Since the amount of BAIBA normally circulating in the blood is so small, the chemical differentiation between physiological and pathological fluctuation is difficult. It was therefore considered of interest to artificially increase the plasma concentration of BAIBA by injecting this substance and to study its plasma clearance before and after irradiation. Under these circumstances, provided that the renal clearance is exceeded, then the rate of disappearance of BAIBA from the plasma is a reflection of metabolic impairment (Smith, Wallas and Sturrock, 1966).

Consequently a third animal experiment was carried out in which two groups of ten rats were each given 10 mg. of BAIBA intraperitoneally. One group served as a control, the other had been irradiated twenty-four hours previously (650 rad × total-body, 250 kV at 15 mA, 1 mm

Table 6.8 Influence of Irradiation upon BAIBA Metabolism in Rat

Changes in plasma BAIBA Concentration

	Time after administration of 10 mg BAIBA i.p. (in mins.)				
	0	30	60	120	180
Control	0.03 ± 0.01	1.33 ± 0.15	0.40 ± 0.03	0.15 ± 0.05	0.10 ± 0.01
Irradiated	0.03 ± 0.01	2.22 ± 0.20	0.52 ± 0.05	0.42 ± 0.03	0.08 ± 0.01

All results in mg per 100 ml plasma ± S.D.

Cu/1 mm. Al filter, dose rate 60 rad per minute). In the three hour period after administration of BAIBA, samples of blood were obtained and the plasma analysed. A significant difference between control and irradiated animals can be observed thirty minutes after an intraperitoneal injection of BAIBA (Table 6.8).

Preliminary studies in rat with C^{14} labelled forms of thymine have shown that irradiation does not affect the catabolism of thymine to BAIBA but that the subsequent degradation of BAIBA is partially inhibited (Bates, Smith and Smith, 1964). This blockage was considered to be as a result of the inhibition or inactivation of the pyridoxal phosphate-dependant transaminase which is involved in the formation of methylmalonic semialdehyde from BAIBA (Figure 6.). It is therefore suggested that a metabolic block may be demonstrated after a loading dose of BAIBA, although the possibility of increased membrane permeability after exposure to radiation cannot be ignored.

This experiment serves to emphasize the value of loading with a particular metabolite in order to saturate the metabolic pathway. This technique is widely used in clinical biochemistry to demonstrate early or a mild form of disease and it is suggested that sub-clinical changes due to radiation may be demonstrable with such a

Table 6.9 Urinary β-Amino Isobutyric Acid (mg/24 hr)

Subject	Control				Sham - Irrad.		X - Irrad.	
N. B. M.	11.4		12.5		-		62.3	
A. F.	8.14		7.76		-		10.05	13.1
W. E.	12.0	12.5	11.6	13.4	-		14.6	
C. M.	5.26		4.36		-		12.5	14.3
A. M. V.	6.05		9.9		-		12.0	19.7
Mc. M.	14.4				16.2		23.6	
J. Na.	6.47				4.8		15.2	
J. W. P.	9.1	9.3	10.0		7.1	7.5	-	
D. W. B.	13.7		13.2		8.36	13.7	-	
H. S. P.	8.5	5.56	4.42	9.6	4.23		-	

Whole Body Irradiated and Controls (15-50 Rads)

	Control	Sham - Irrad.	X - Irrad.
Mean	9.5	8.8	19.7
Mean % Change	-	- 8 %	+ 107.0%
Number of Observations	23	7	10
High	14.4	16.2	62.3
Low	4.42	4.23	10.05

technique.

6.4 Whole-Body Exposure Studies

During the course of these investigations a series of patients acutely exposed to whole-body irradiation were studied.

Unfortunately, no plasma investigations were made, but urine samples from some ten patients exposed to 15-20 rad whole-body were studied. Samples were obtained after sham-irradiation treatment as well as after exposure to low doses of whole-body X-irradiation and controls.

(i) BAIBA

The results obtained for BAIBA are shown in Table 6.9. Even at the low levels of radiation involved, there would appear to be quite a significant increase in urinary BAIBA after exposure to radiation, and, more important, that this increase is not due in any psychological way to the type of treatment involved. In this respect, Berry has reported (1960) that in some instances, excretion of BAIBA may represent a reaction to stress. Comparing the figures to those obtained from the initial partial body studies, it would appear that 15-50 rads whole body exposure, is if anything, more effective in producing a biochemical lesion that 150-300 rads partial body

exposure in the abdominal region. This perhaps serves to emphasize the limitation of partial body exposure studies.

(ii) Phenylalanine and Tyrosine

In conjunction with the whole-body exposure BAIBA studies, a study of the urinary levels of tyrosine and phenylalanine in the same series of subjects was made. The reason for this was two-fold.

(i) Utilisation of the special chromatographic system for rapid BAIBA determination was also very suitable for the specific and accurate integration of these two amino acids which occur in the same region of the chromatogram as BAIBA, and during the course of BAIBA studies, small fluctuations in the peaks of tyrosine and phenylalanine were apparent. Therefore it seemed of interest to investigate them.

(ii) It has been reported (Ross and Ely, 1951) that in animals there is an increase in liver weight after exposure to radiation, and further that this increase is mainly due to an increase in liver glycogen (Supplee et al., 1954). It is possible that this increased glycogen is due, at least in part, to the increased

Table 6.11 Whole Body Irradiated and Controls

Urinary Phenylanine (μM/24 hr.)

Subject	Control				Sham-Irrad.		X-Irrad.*	
N.B.M.	20.3		36.7		-		34.5	
A.F.	93		64.3		-		71.5	106
W.E.	49.1	51	41.9	53.5	-		68	
A.M.V.	38.6		87.2		-		82.2	102.5
Mc.M.	127				170		185	
J. Na.	100				89.5		139	
J.W.P.	56.3	53	51		47.2	33.2	-	
D.W.B.	72.5		82		53.3	66.2	-	
H.S.P.	78.5	95	89	94.3	69.7		-	

*15-50 rad whole body

	Control	Sham-Irrad.	X-Irrad.
Mean	68.3	75.5	98.6
Mean % change	-	+ 10 %	+ 45 %
Number of Observations	21	7	8
High	127	170	185
Low	20.3	33.2	34.5

Table 6.10 Whole Body Irradiated and Controls

Urinary Tyrosine (μM/24 hr.)

Subject	Control				Sham-Irrad.		X-Irrad.*	
N.B.M.	109		118		-		166	
A.F.	186		205		-		160	238
W.E.	105	114	85	105	-		122	
A.M.V.	90		168		-		183	267
Mc.M.	369				433		523	
J.Na.	173				131		211	
J.W.P.	64.5	61		62.7	60.5	48.8	-	
D.W.B.	195		115		71	112	-	
H.S.P.	124	147	145	150	105		-	

*15-50 rad whole body.

	Control	Sham-Irrad.	X-Irrad.
Mean	138	137	234
Mean % change	-	-	+ 70%
Number of Observations	21	7	8
High	369	433	523
Low	61	60.5	122

Range (from literature) 15-45 mg/24 hr.

availability of glycogenic amino acids released into the metabolic pool after exposure to radiation and subsequent tissue and cellular damage. Most of the naturally - occurring amino acids are considered to be glycogenic and this possibility of glycogen formation from the excess amino acids released after exposure to ionising radiations could account for the absence of a general amino-aciduria in the urine after exposure to low doses of X-irradiation. There are only three or four natural amino acids which are considered to be ketogenic, and two of these are tyrosine and phenylalanine. Since the ketogenic amino acids cannot be utilised in the formation of liver glycogen it might be possible that there could be increased ketogenic amino acids in the urine in the absence of increased glycogenic amino acids after exposure to X-irradiation.

Accordingly an investigation of urinary tyrosine and phenylalanine levels was carried out. The results are shown in Tables 6.10 and 6.11. Although there would appear to be a trend towards increased amounts of both metabolites in the urine following exposure

to X-irradiation, with the exception of the tyrosine levels for one patient, namely McM., which were above the normal range throughout, there was no increase of either metabolite outwith the normal daily range. However, this increase in both metabolites might be a reflection of some biochemical lesion resulting from radiation damage, and therefore well worthy of further study in the future. The possibility of a renal factor cannot be ignored since relative plasma studies were unfortunately omitted.

6.5 Loading Function Experiments : First Series

From a consideration of the results of the second rat experiment (Table 6.8), a defect in the clearance of loaded BAIBA was obvious after irradiation. This type of function test was now applied to human studies.

Steric Considerations

Since the α carbon atom of BAIBA is asymmetric, the question of optical isomerism should be considered in treating the metabolism of BAIBA, and particularly in BAIBA loading function tests. BAIBA from the urine of genetic excretors has been shown to be laevorotatory (Crumpler et al., 1951). However, it has been observed by Fink, Henderson and Fink (1952) that only 15% of a racemic mixture of BAIBA injected intra-peritoneally into

Table 6.12 BAIBA Clearance from Rabbits

Rabbit 6b. Same 3 Kg. Rabbit. Loaded 150 mg DL BAIBA i.v. one hour after exposure to 1000 rad whole body.

PLASMA BAIBA (mg/100 ml)				URINARY BAIBA (mg/sample)			
1.	7.24	at	30 mins.	1.	11.50	at	30 mins.
2.	2.00	at	60 mins.	2.	4.85	at	60 mins.
3.	0.57	at	120 mins.	3.	3.71	at	120 mins.
				4.	0.09	at	180 mins.

Rabbit 7. 2.2 Kg. Rabbit. Loaded 110 mg DL BAIBA i.p. Rabbit was nephrectomised.

PLASMA BAIBA (mg/100 ml)			
1.	11.80	at	30 mins.
2.	4.27	at	60 mins.
3.	0.85	at	120 mins.
4.	1.02	at	180 mins.

Plasma values in mg/100 ml.

Urine values in mg/sample.

All times represent time at which sample was taken after loading animal with DL BAIBA.

Table 6.12 BAIBA clearance from Rabbits

Rabbit 4. 2.75 Kg. Rabbit. Loaded 28 mg BAIBA + 10 mg Thymine.

PLASMA BAIBA (mg/100 ml)			
1.	3.22	at	10 mins.
2.	0.62	at	30 mins.
3.	0.46	at	60 mins.
4.	0.15	at	120 mins.
5.	0.10	at	180 mins.
6.	0.10	at	240 mins.
7.	0.10	at	300 mins.
8.	0.07	at	360 mins.

URINARY BAIBA

Total urinary BAIBA excreted in 6 hours was 1.29 mg.

Rabbit 5. 2.8 Kg. Rabbit. Loaded 39.2 mg BAIBA + 10 mg Thymine.

PLASMA BAIBA (mg/100 ml)			
1.	0.10	at	0 mins.
2.	2.23	at	10 mins.
3.	0.57	at	30 mins.
4.	0.22	at	60 mins.
5.	0.14	at	120 mins.

URINARY BAIBA

Total urinary BAIBA excreted in 6 hours was 11.6 mg.

Rabbit 6a. 3 Kg. Rabbit. Loaded 150 mg DL BAIBA i.v.

PLASMA BAIBA (mg/100 ml)			
1.	13.5	at	30 mins.
2.	4.54	at	60 mins.
3.	0.257	at	120 mins.

URINARY BAIBA (mg/sample)			
1.	16.4	at	30 mins.
2.	8.0	at	60 mins.
3.	1.0	at	180 mins.

Table 6.12 BAIBA clearance from Rabbits

Rabbit 1. 2 Kg. Rabbit. Loaded 28 mg DL BAIBA monohydrate i.v.

PLASMA BAIBA (mg/100 ml)			URINARY BAIBA (mg/sample)		
1.	8.3	3 mins. after loading	1.	0.161	at 5 mins.
2.	0.735	at 30 mins.	2.	0.528	at 35 mins.
3.	0.158	at 60 mins.	3.	0.113	at 60 mins.
			4.	0.124	at 120 mins.
			5.	0.028	at 180 mins.

Rabbit 2. 2.1 Kg. Rabbit. Loaded 28 mg DL BAIBA monohydrate. Received 1000 rads whole body.

PLASMA BAIBA (mg/100 ml)			URINARY BAIBA (mg/sample		
1.	9.3	at 3 mins.	1.	0.059	at 8 mins.
2.	0.816	at 30 mins.	2.	0.0065	at 35 mins.
3.	0.473	at 60 mins.			
4.	0.26	at 120 mins.			
5.	0.966	at 180 mins.			

Rabbit 3. 2.4 Kg. Rabbit. Loaded 28 mg BL BAIBA monohydrate.

PLASMA BAIBA (mg/100 ml)			URINARY BAIBA
1.	1.87	at 10 mins.	Total urinary BAIBA excreted in 6 hours 20 mins. was 2.54 mg.
2.	0.286	at 30 mins.	
3.	0.323	at 60 mins.	
4.	0.175	at 120 mins.	
5.	0.156	at 180 mins.	

rats is recovered in the urine. This would suggest that both forms can be metabolised. The results obtained in the rabbit experiments previously described confirm Fink's observations in this species. (Table 6.12).

There is at present only the racemic form of BAIBA available commercially. This is unsuitable since there may well be two quite different metabolic systems for the different isomers (Gartler, 1960). What is required therefore, is a source of the natural L-isomer. (The nomenclature adopted here is L = laevorotatory; D = dextrorotatory). The isolation of this natural isomer from human urine is a long and difficult process (Kakimoto and Armstrong, 1961), and in order to obtain sufficient quantities for function tests, large volumes of urine from a genetic high BAIBA excretor would be required. The reason that much BAIBA is required for loading experiments is threefold:-

(i) There appears to be a very low renal threshold for BAIBA in the normal individual. Therefore it is difficult to saturate the metabolic system.

(ii) BAIBA has a very low colour yield with ninhydrin. This colour yield is approximately three times less than that for most of the

other common naturally-occurring amino acids. Hence detection in physiological fluids can be difficult.

(iii) Normal humans would appear to be able to deal very efficiently and rapidly with BAIBA, as it is normally very quickly metabolised in the body. Hence it is necessary to attempt to saturate the metabolic system in question, in order that any biochemical lesion resulting from radiation exposure will become more readily discernable. Loading experiments at present being carried out on rabbits indicate an even more rapid and efficient metabolism of BAIBA in this species.

However another source of natural BAIBA is readily available. Fink and her associates have established that the pyrimidine base of DNA, thymine, is a precursor of BAIBA, and in fact is probably the main source of BAIBA in mammals. (Fink, Henderson and Fink, 1952; Fink, Fink and Henderson, 1952; Fink, McGeughey, Cline and Fink, 1956; Fink, 1956). This has been confirmed by Awapora and Shullenberger (1957). Therefore an experimental scheme was developed whereby patients were loaded with thymine, orally, before and after receiving therapeutic doses of

Table 6.13 Thymine Loaded Subjects

Urinary β-Aminoisobutyric Acid (μM/24 hr)

Subject	Pre	Thymine Load	Thymine Load Day 1	Thymine Load Day 2	Thymine Load Day 3
		+	+	+	+
I.Mc.D.	72	547	1957	1931	2464
O.M.H.	250	912	1053	690	520
T.G.W.	157	910	1565	1240	1440
G.S.H.	465	740	3190	1685	-
D.W.*	967	7500	6060	1560	3060
D.Mc.K.*	2170	4850	6900	4755	-
E.D.C.	196	693	891	736	660
A.F.O.	128	1130	940	646	1079
S.J.S.	90	2285	4433	2645	2068
J.H.R.	53	844	826	437	-
Mean*	178	1008	1857	1251	1372
Mean % Change	-	+ 466%	+ 945%	+ 600%	+ 670%
No. of Obs.	8	8	8	8	8

*excluding high excretor.

+subjects given 500 mg thymine orally each day; on day 1, given thymine immediately after X-irradiation therapy.

Table 6.14 Plasma β-Aminoisobutyric Acid (μM/100 ml)

Subject	C	C + 3 hr	C + 24	X + 3	X + 12	X + 24	X + 27	X + 48	X + 51	X + 72
D.W.	1.77	5.50	0.70	4.44	0.57	0.30	2.64	0.57	2.30	-
T.G.W.	0.54	2.35	-	2.66	1.58	0.40	2.72	0.87	1.78	1.28
O.M.H.	0.60	0.20	-	2.52	0.30	0.85	1.10	0.76	0.80	0.72
G.S.H.	1.90	3.40	1.14	1.54	-	1.24	2.40	4.51	-	-
D.Mc.K.	0.30	3.32	-	4.23	0.37	0.33	5.28	1.04	-	-
I.Mc.D.	1.40	0.80	-	1.30	-	0.77	1.55	1.94	1.57	1.61
MEAN	1.08	2.60	0.92	2.78	0.70	0.65	2.61	1.61	1.61	1.20
HIGH	1.90	5.50	1.14	4.44	1.58	1.24	5.28	4.51	2.30	1.61
LOW	0.30	0.20	0.70	1.30	0.30	0.30	1.10	0.57	0.80	0.72
No. of Obs.	6	6	2	6	4	6	6	6	4	3

C - Control

X - Irradiated

Subjects given 500 mg Thymine orally immediately after C, X, X+24, X+48.

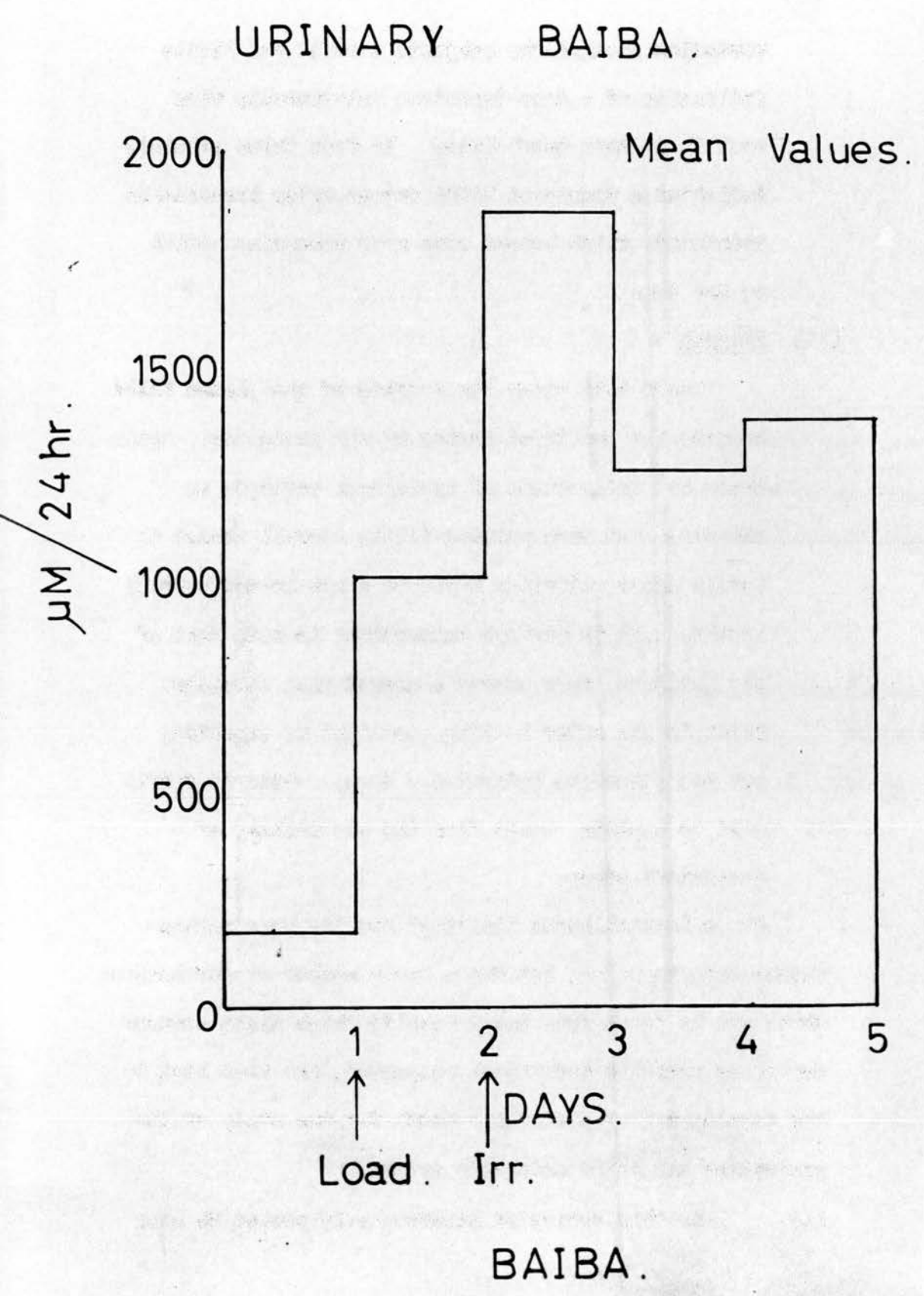
partial-body X-irradiation.

Initially a series of six patients was taken, each one receiving prophylactic abdominal irradiation. They were exposed to the same dose of partial-body X-irradiation (150 rad.) and urine samples were collected for 24 hour periods. 500 mg of thymine; equivalent to 400 mg BAIBA on a molecular weight basis, was administered orally by capsule, one every 24 hours. On the day of irradiation, the capsule was given immediately after radiation treatment. Twenty-four hour urine samples and ten plasma samples throughout the five day period were obtained and analysed specifically for BAIBA. The results are shown in tables 6.13 and 6.14.

(i) Urines

Table 6.13 includes the results of the total 24 hour urine samples from the next four subjects. The marked increase in urinary BAIBA levels following thymine loading confirms beyond all doubt that thymine is an important precursor of BAIBA. The amount of BAIBA in the urine increased, on average, by about 450% after oral administration of 500 mg thymine. On the day following exposure to X-irradiation the mean figure indicated a general increase in BAIBA excretion (Figure 10), but there was a wide individual

Fig. 10


URINARY BAIBA.
Mean Values.
2000
1500
1000
500
0
µM/24 hr.
1
2
3
4
5
DAYS.
Load.
Irr.
BAIBA.

variation amongst the subjects studied and little indication of a dose-dependant relationship with regards to this metabolite. In fact three patients indicated a decreased BAIBA output after irradiation treatment, which became even more pronounced still on day two.

(ii) Plasmas

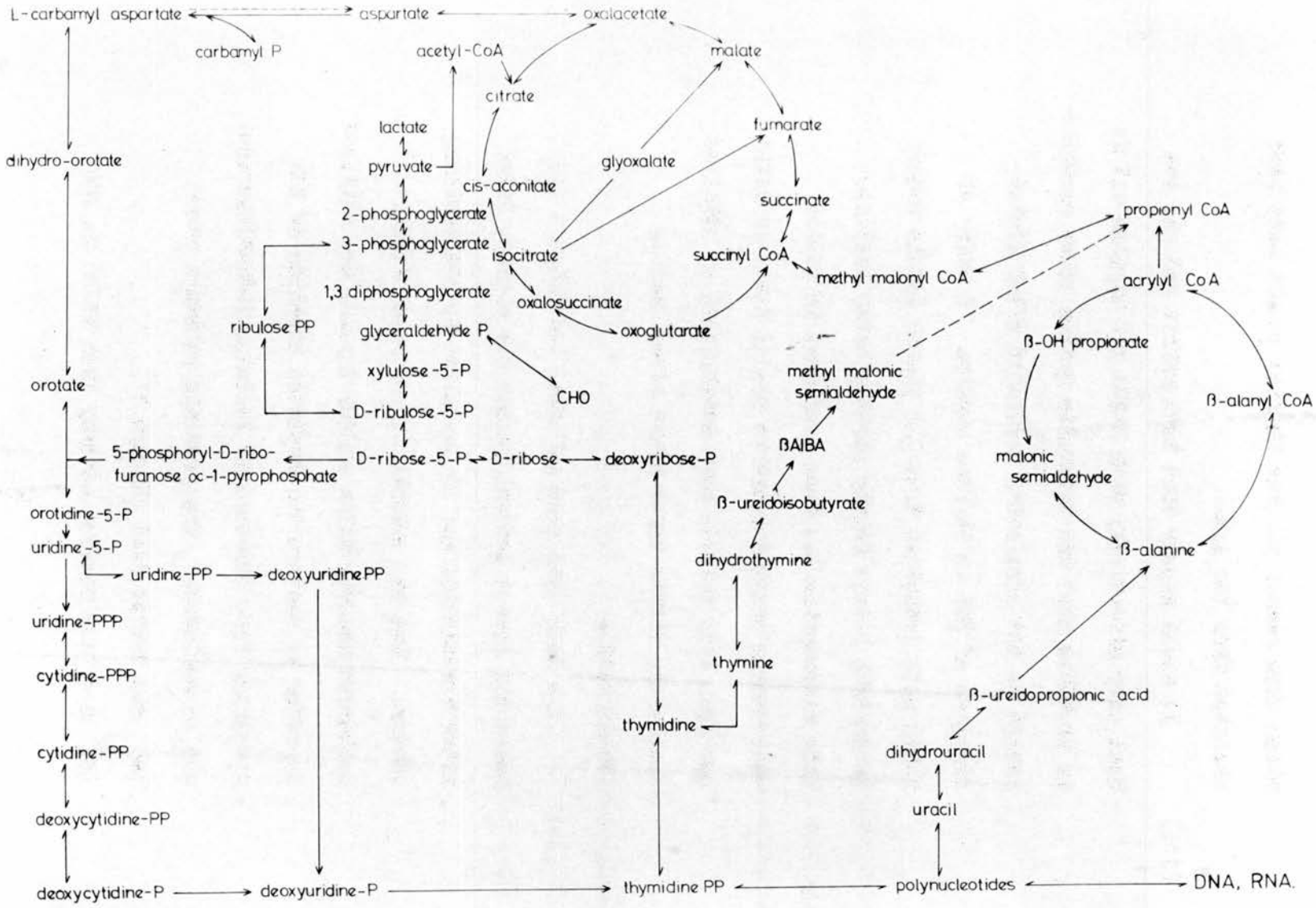
Table 6.14 shows the results of the plasma BAIBA studies for the first series of six patients. Again there are indications of individual variance in response, but with perhaps little overall change in levels after radiation exposure above loaded control levels. It is perhaps interesting to note that of six patients, four showed a marked rise in plasma BAIBA levels after loading, as might be expected, but the other two indicated a drop. This is difficult to explain, apart from the possibility of analytical error.

These initial human "loading" studies were perhaps rather disappointing, but there are a number of conclusions which can be drawn from these results which might account for these variable individual responses, and thus lead to the development of a suitable model for the study of the pyrimidine and BAIBA metabolic system.

(i) In this series of studies, only pooled 24 hour

urine samples were analysed, due to limited analytical resources. Function tests in the rabbit have revealed that there is a very rapid turnover of BAIBA after loading and that the levels of this metabolite are back to control values within about 3-4 hours (Table 6.12). It is probable that the same state of affairs is to be found in the normal human. Hence any increase in the levels of BAIBA after either loading or X-irradiation would probably be a short-term effect only. Consequently the necessity for the analysis of time-fractionated urine samples was indicated.

(ii) It is well known that exposure of a human to X-irradiation causes vomiting (Hempelman, Lisco and Hoffman, 1952; Wald and Thoma, 1961). Some of the subjects studied in this series of investigations did vomit, and since the thymine was administered orally immediately after radiation treatment some of the administered thymine could have been lost. This is one explanation for the wide individual variations in response, since all the loaded patients received exactly the same radiation treatment. To overcome this vomiting factor, it was decided to administer the thymine about 2 hours before radio-therapy, by



PYRIMIDINE BIOSYNTHESIS & CATABOLISM.

Fig. 11

which time nearly all the thymine should have been absorbed from the gut.

(iii) It would appear that both rabbit and man can deal very effectively with BAIBA and thymine and it is probable that the metabolic system under consideration is not completely saturated after administration of 500 mg thymine orally. A study of Table 6.14 indicates that the plasma levels return to control levels fairly rapidly after loading. This observation has been confirmed in rabbit experiments where comparable loaded doses of BAIBA per Kg., body weight, were metabolised or utilised completely within three hours after loading (Table 6.12).

(iv) The fact that some subjects indicated a drop below the loaded control values for urinary BAIBA after irradiation can be explained by the vomiting factor. But the possibility of some "feed-back" mechanism whereby BAIBA and/or thymine are utilised in order to restore the depleted supplies of DNA resulting from exposure to ionising radiations cannot be excluded. Two possible pathways are:-

(a) via Orotic Acid (Figure 11),

(b) a direct reverse pathway from BAIBA to DNA

Table 6.15 Thymine Loaded, Non-Irradiated Controls

URINARY β-amino-isobutyric Acid (mg/6 hr. period)

SUBJECT	AR	AL	RF	MEAN
Control Urine	1.5	16.4	10.9	9.6
Urine Volume	391	380	308	360
Thymine Load 1	82.3	110.8	37.5	76.9
Urine Volume	359	286	266	304
Thymine Load 2	55.8	127.7	47.0	76.8
Urine Volume	234	320	276	277

Normal Volunteers.

Urine collections for 6 hr. period, after loading with 500 mg. thymine orally.

via thymine. The existence of the latter pathway has not yet been established, and it is planned, in the future, to study this system in an attempt to discover if in fact it exists.

(v) The possibility of a renal factor in relationship to increased urinary BAIBA cannot be ignored. To establish this fully, it is necessary to obtain blood samples at regular intervals throughout the study, but the number which can be withdrawn from any one subject per day is, of course, limited.

6.6 Control Loaded Subjects

The next logical step was to obtain some control information on the effect of thymine loading with regard to urinary BAIBA levels in the absence of X-irradiation. Table 6.15 shows the results for three control volunteer individuals.

Admitting a paucity of statistical evidence, it seems probable that there is little or no build up of thymine resulting from loading over two consecutive days. Thus any significant rise above loaded control levels after irradiation is, in the main, due to a metabolic lesion resulting from exposure to ionising radiation, and not simply a gradual accumulation of BAIBA in the tissues resulting from continual thymine loading.

Table 6.17 Plasma β-Aminoisobutyric Acid (μM/100 ml)

Subject	C	C+1 hr	C + 3	X + 1	X + 3	X + 25	X + 27	X + 49	X + 51
A.F.O.	0.70	0.65	1.65	2.36	1.70	0.71	1.10	1.72	1.14
S.J.S.	1.12	3.21	5.23	4.22	3.94	2.04	4.21	1.48	3.45
E.D.C.	0.37	1.11	1.04	1.84	0.50	2.94	0.89	1.88	1.16
J.H.R.	0.51	1.55	1.46	3.77	2.90	2.61	2.19	-	-
Mean	0.67	1.63	2.34	3.05	2.26	2.07	2.10	1.69	1.92
High	1.12	3.21	5.23	4.22	3.94	2.94	4.21	1.88	3.45
Low	0.37	0.65	1.04	1.84	0.50	0.71	0.89	1.48	1.14
No. of Obs.	4	4	4	4	4	4	4	3	3

C - Control

X - Irradiated

Subjects given 500 mg Thymine orally immediately after C, X, X + 24, X + 48.

Table 6.16 Urinary β-Aminoisobutyric Acid (mg/sample)

Sample No.	Sampling Time (hr)	Subject				Mean
		E.D.C.	A.F.C.	S.J.S.	J.H.R.	
1	24	19.60	12.80	9.00	5.34	11.68
2	2	19.10	14.00	39.50	20.90	23.37
3	2	44.70	64.00	141.30	53.30	75.82
4	4	2.90	34.40	45.00	2.42	21.18
5	16	22.60	0.64	2.73	7.80	8.94
6	2	42.50	29.20	107.00	39.60	53.08
7	2	20.60	57.30	230.00	24.80	83.17
8	4	4.50	4.77	98.70	7.62	28.90
9	16	21.50	2.30	7.57	10.60	10.49
10	2	38.70	2.33	38.20	13.70	23.23
11	2	15.20	20.50	169.50	23.10	57.32
12	4	3.77	40.00	55.30	2.64	25.43
13	16	15.90	1.80	1.49	4.29	5.87
14	2	18.00	35.90	38.00	-	30.63
15	2	31.80	63.00	126.00	-	73.60
16	4	3.96	8.32	40.60	-	17.63
17	16	12.80	0.70	2.17	-	5.22

Subjects given 500 mg Thymine orally, after samples 1, 5, 9, 13.

Subjects received X-irradiation (300 rads) between samples 5 and 6.

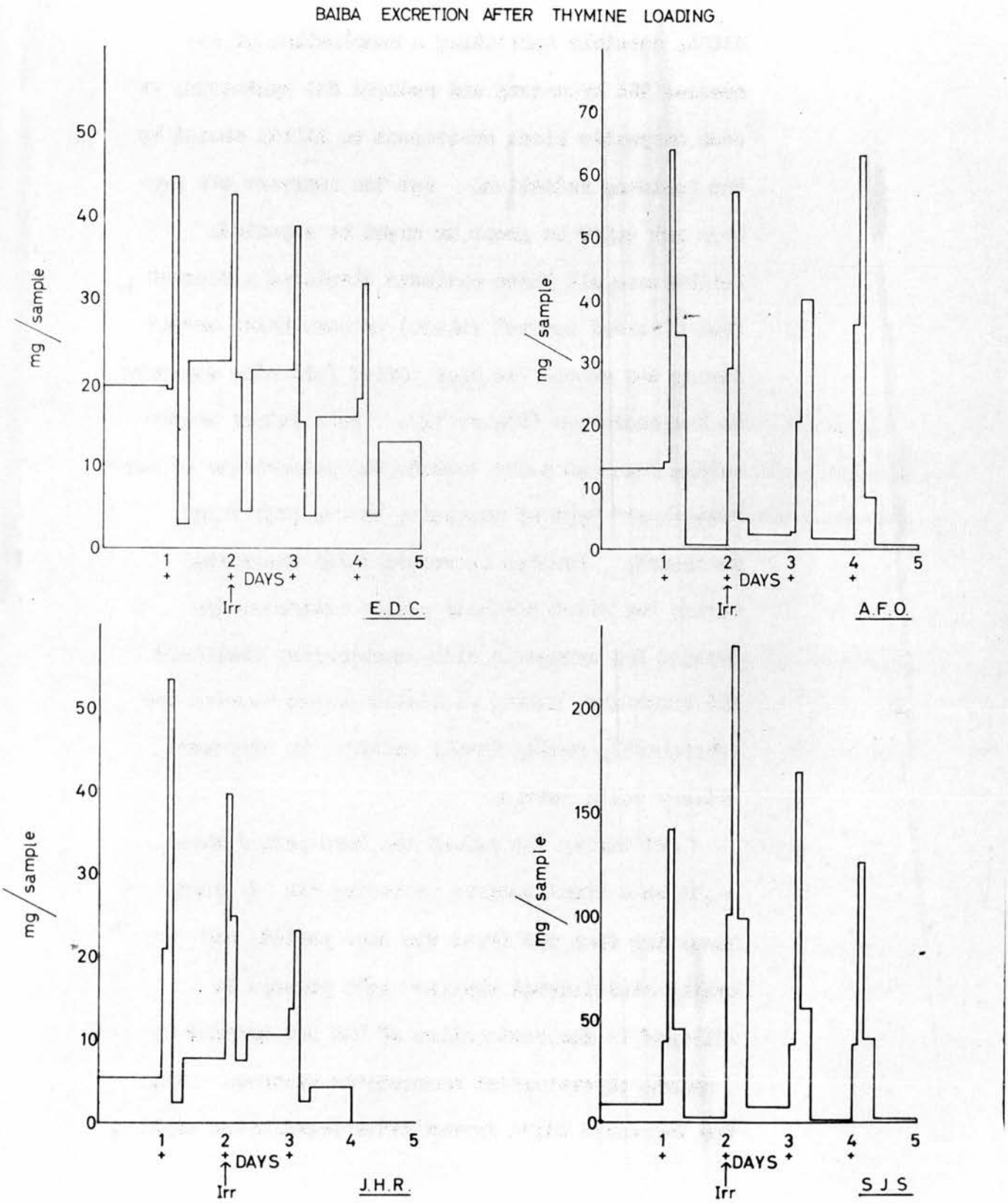
6.7 Loading Function Experiments : Second Series

A further series of four thymine-loaded patients was now studied in which the daily urine samples were time-fractionated into 2, 2, 4 and 16 hours after loading and irradiation. This was carried out in order to establish if there was a very rapid turnover of the administered thymine in man. Plasma samples were also obtained at 1 hour after loading each day. Otherwise the treatment was exactly the same as with the first series of loaded patients. i.e. thymine was given immediately after radio-therapy. The results are shown in Tables 6.16 and 6.17. As was expected, most of the BAIBA excreted appeared in the urine during the first 4 hours after loading, emphasising the necessity of multi-sampling during the first 6 or 8 hours following oral administration of thymine. But while three of the patients would appear to show a more or less similar response, the fourth, namely S.J.S., was quite different.

(i) Urine

The response of three patients all seemed to indicate the same pattern, but it was not quite as expected. In the first two hour period following exposure to radiation there was the expected increase, above the loaded control values, in urinary

Fig. 12


BAIBA EXCRETION AFTER THYMINE LOADING
mg/sample
DAYS
Irr
E.D.C.
A.F.O.
J.H.R.
S.J.S.
+ = 500mg THYMINE

BAIBA, possibly indicating a combination of increased DNA breakdown and reduced DNA synthesis, or some enzymatic block subsequent to BAIBA, caused by the ionising radiation. But the increase was perhaps not quite so great as might be expected. Furthermore all three patients displayed a reduced (below loaded control values) urinary BAIBA output during the second two hour period following exposure to X-irradiation (Figure 12). This latter observation seems to point towards the possibility of some "feed-back" type of mechanism as was previously mentioned. Perhaps increased BAIBA excretion during the first two hour period indicates decreased DNA synthesis with accompanying increased DNA breakdown, making available excess thymine and subsequently making itself manifest in increased urinary BAIBA levels.

But during the second two hour period there might be a trend towards restoring the DNA debt resulting from the first two hour period, and the orally administered thymine might perhaps be utilised in the restoration of the DNA balance by a normal physiological homeostatic process. Or, the increased BAIBA formed after irradiation might

be used, via aspartate and the orotic acid cycle, to restore the DNA balance.

But the fourth patient, S.J.S., showed a quite different pattern and very marked response to X-irradiation. There was a large increase during the first two hour period and an even more marked increase during the second two hour period, which was in direct contradiction to the response of the other three subjects. However there were certain irregularities in this particular case. S.J.S. was an old man of about 70 and unlike the others did not vomit. Therefore he would not lose any thymine through vomiting, whereas much of the orally administered thymine may have been lost in this way after radio-therapy in the other cases.

(ii) Plasma

The plasma levels of the three patients exhibiting the similar excretory pattern were also much alike, and all seemed to indicate a tendency to elevated plasma BAIBA levels after exposure. If considered in conjunction with the second two hour lowering of urinary BAIBA output, this factor may be indicative of increased renal re-absorption. S.J.S. while exhibiting high plasma levels throughout remained fairly constant before and after irradiation.

This series proved conclusively that the BAIBA response to low-level irradiation is a relatively short-term effect and stressed the need for multi-sampling during at least the first 8-hour period after either loading or irradiation, or both. Also it is most likely that the difference in response between S.J.S. and the other three cases results from the vomiting factor, but at the same time it seems indicative from the control loaded levels that S.J.S. exhibits a much less efficient ability to metabolise BAIBA in the event of its excessive production.

6.8 Loading Fuction Experiments : Third Series

It has been stated that an unknown amount of the orally administered thymine may be lost through vomiting subsequent to radio-therapy. The next logical step was to endeavour to overcome this limiting factor. The two final patients in the present series of investigations were given the thymine orally two hours before radiation treatment, by which time nearly all the administered thymine should have passed from the stomach. The urines were time-fractionated as in the previous group.

(i) Urine

The results are shown in Table 6.18. Both

Table 6.18 Urinary β-Aminoisobutyric Acid (mg/sample)

Sample No.	Sampling Time (hr)	Subjects		Mean	24 hr. Mean
		R.J.D.	J.H.		
1	24	9.10	15.0	12.05	12.05
2	2	7.05	6.96	7.00	74.92
3	2	26.60	59.60	43.10	
4	4	10.10	32.40	21.35	
5	16	4.47	2.68	3.57	
6	2	2.34	17.60	9.97	218.57
7	2	66.50	173.00	119.75	
8	4	85.30	76.80	81.05	
9	16	11.90	3.71	7.80	
10	2	20.20	8.00	14.10	88.02
11	2	34.40	52.50	43.45	
12	4	11.30	36.00	23.65	
13	16	9.30	4.34	6.82	

Subjects given 500 mg. thymine orally, after samples 1, 5 and 9.

Subjects received partial body X-irradiation (300 rad.) between samples 6 and 7.

subjects showed a marked response with a 3 fold increase in urinary BAIBA levels above control loaded levels after exposure to radiation. These observations indicate that pre-irradiation loading definately obviates oral thymine loss through vomiting.

(ii) Plasma

The results of the plasma BAIBA determinations are shown in the following table.

Table 6.19 Plasma β-aminoisobutyric acid (μM/100 ml.)

Subject	C	C + 3	X + 3	X + 27
R.J.D.	0.78	2.40	5.02	1.58
J.H.	0.65	3.79	6.00	1.93

C - Control

X - Irradiated

Blood samples were withdrawn 3 hours after oral loading with 500 mg. thymine in each case.

A response similar to that of the urinary BAIBA pattern was observed. There was about a 2 fold increase in the plasma BAIBA levels above the control loaded levels, 3 hours after exposure to radiation.

This is the type of response which is required as a sensitive and early biological indicator of

radiation induced changes in man. However, many more subjects must be studied in the future, using as a basis this technique of pre-irradiation oral administration of thymine, before any possibility of a dose-dependant BAIBA response can be established.

It is indicated that the development of a suitable model for the investigation of the response of the DNA pyrimidine metabolic pathway to ionising radiation is progressing on the right lines, but more studies are required involving the following:-

(i) Renal Effects: The possibility of an alteration in renal function as a result of radiation, with subsequent variation in urinary metabolites must be investigated. It is well-established that the urine output is generally decreased immediately after exposure to radiation and if there is a significant alteration this factor will tend to preclude the possibility of the adoption of an urinary metabolite variation as a biological indicator of absorbed dose.

(ii) The possibility of feed-back mechanisms and other associated metabolic pathways (e.g. BAIBA → Thymine) and their response to

radiation should be investigated. It is quite possible that these mechanisms are indirectly stimulated by the body's response to radiation, and as a result of their stabilising action, tend to obscure the biochemical lesions caused by radiation.

(iii) It is possible that saturation of the specific metabolic system has not been achieved. It is desirable to accomplish this either by increasing the oral thymine dose or by establishing a suitable block in the metabolic pathway. For example, in the Krebs cycle by malonate inhibition of succinic dehydrogenase.

(iv) Thymine studies must be carried out using C^{14} labelled thymine (in animals) in order to ascertain the fate of the administered thymine.

(v) Intraperitoneal injection of both thymine and BAIBA, is required to obviate any loss through oral loading.

(vi) Animal studies to determine the kinetics of the pyrimidine pathway and the exact site of major BAIBA metabolism should be carried out.

7. POSSIBLE RENAL FACTOR

7.1 Introduction

As can be seen from the results of the foregoing BAIBA studies there would appear to be a wide variation in the individual response to exposure to ionising radiation in both the "loaded" and "non-loaded" subjects. One of the major aims in the present study is to endeavour to develop a suitable model for the study, in the human being, of the effect of radiation on DNA and related pyrimidine metabolic pathways, and to this end, an attempt has been made to eliminate the accompanying variable parameters.

In the light of further studies, particularly those involving animals, it has become increasingly apparent that it is probably the plasma metabolite levels which are of greatest importance. Unfortunately, in the present work these were largely neglected, and most of the results are concerned with urine analysis. In the plasma changes are of a smaller magnitude than in urine but it may well be that these subtle plasma metabolite variations are of much greater importance. However they suffer from the defect of the requirement of highly refined analytical techniques for accurate detection and quantitation.

This was the reason for the 'loading' function tests

Table 7.1 18 Patients: - Non-Loaded - 24 hr. Samples

Comparison of Urinary BAIBA Output and Related Urine Volume

Subject	Pre		Day 1		Day 2		Day 3		Day 4	
	BAIBA	Urine Vol.	BAIBA	Urine Vol.	BAIBA	Urine Vol.	BAIBA	Urine Vol.	BAIBA	Urine Vol.
Testicular Tumours										
J.Mc.C.	76	1500	117	1156	146	1290	175	1290	-	-
R.W.C.	172	1790	82	1121	53	1450	111	2000	134	1800
R.S.*	2290	1270	2470	910	940	1090	1660	1100	2160	1600
J.T.M.	204	1290	128	1020	260	1580	53	1640+	-	-
R.S.C.	140	1580	207	952	193	1010	178	1280	-	-
J.R.N.*	576	2720	866	960	595	1140	394	2100	-	-
J.S.	129	2350	50	1930	205	524	143	2160	59	1710
A.M.	175	1930	318	3010	132	657	168	883	-	1160
J.R.B.	310	1500	327	1215	406	630	286	745	199	700
A.B.	67	1820	93	2820	117	2710	56	1910	26	2220
N.B.B.	47	1360	100	1145	67	765	50	853	73	1130
J.D.M.	120	1405	166	2330	213	1960	187	820	120	1335
D.C.R.	72	1400	87	945	269	645	206	564	330	700
A.J.	82	1690	168	848	219	1185	256	1265	189	1015
Spondylitics										
T.C.B.	41	1040	115	1510	-	-	-	-	-	-
W.T.	114	1325	125	1964	414	1750	166	1620	-	-
W.B.	122	1550	190	1674	257	1330	353	1460	-	-
M.M.*	3910	1098	4000	1730	3110	1520	2850	1640	1976	575
M.C.	174	800	188	616	222	1240	178	1170	-	-
S.B.	120	1180	234	1090	168	1540	113	1780	219	1740
J.K.	15	1300	103	520	133	720	149	540	201	770
Mean*	121	1489	155	1437	207	1234	166	1293	174	1586
Mean % Change*	-	-	+ 28%	-3.6%	+ 71%	- 20%	+ 37%	- 15%	+ 44%	+6.5%
Low*	15	800	50	520	55	524	30	540	26	700
High*	310	2350	327	2820	414	1960	353	2000	350	2220
	A	B	A	B	A	B	A	B	A	B

A - BAIBA μM/24 hr.

B - Urine volume ml/24 hr.

* - Excluding 3 high excretors.

in an attempt to magnify these small variations in the blood metabolite levels.

7.2 Importance of Clearance Rate

From a consideration of the urine studies, an obvious variable becomes at once apparent. This is the rate and volume of urine flow. The kidney is a major homeostatic mechanism in the body and the amount of re-absorption and hence the amount of a particular metabolite in the urine, is dependent on the clearance rate. It is an established fact that there is a tendency to decreased volume of urine in the few hours immediately post-irradiation exposure. This is as might be expected since, amongst other things, vomiting is a common sympton of the acute radiation syndrome. Therefore any observed changes in various urinary metabolites after X-irradiation exposure might simply be as a result of altered urine flow rate.

It was therefore decided to establish if there was any relationship between the observed urinary BAIBA levels and the related urine volumes.

7.3 Results

Table 7.1 shows the observations for the first series of 18 "non-loaded" patients. The expected decrease in urine volumes subsequent to radiation treatment was observed, but this decrease was not significant.

Table 7.2 Thymine Loaded Patients - 24 hr. Samples

Comparison of Urinary BAIBA Output and Related Urine Volume

Subject	Pre		Thymine Load		Day 1		Day 2		Day 3	
	BAIBA	Urine Vol.	BAIBA	Urine Vol.	BAIBA	Urine Vol.	BAIBA	Urine Vol.	BAIBA	Urine Vol.
			+		+		+		+	
I.Mc.D.	72	1460	547	1710	1957	1960	1931	1940	2468	1820
O.M.H.	230	1375	912	1465	1053	920	690	1320	520	1500
T.G.W.	137	2130	910	2155	1363	1580	1240	1690	1440	1440
G.S.H.	465	935	740	1110	3190	1135	1685	1060	-	-
D.W.	967	1300	7500	1970	6060	945	1560	2180	3060	1520
D.Mc.K.*	2170	1510	4830	2190	6900	1850	4733	2150	-	-
Mean*	382	1440	2122	1682	2765	1308	1421	1638	1872	1570
Mean % Change	-	-	+458%	+ 17%	+624%	- 11%	+265%	+ 14%	+390%	+ 9%
Low	72	935	547	1110	1053	920	690	1060	520	1440
High	967	2130	7500	2155	6060	1960	1685	2180	3060	1820
	A	B	A	B	A	B	A	B	A	B

A - BAIBA μM/24 hr.

B - Urine volume ml/24 hr.

\+ - 500 mg thymine administered orally.

X-irradiation on day 1.

* - excluding high excretor D.Mc.K.

However it must be borne in mind that only total 24 hour sampling was carried out with these subjects, and any immediate significant post-exposure decrease might well be masked in the total 24 hour sample. From the results obtained in this series there would appear to be no obvious relationship between urine volume and BAIBA excretion.

The results of the first series of loaded subjects (Table 7.2) also indicated that there is no apparent relationship between BAIBA output and 24 hour urine volumes. The study was now applied to the urinary time--fractionated thymine-loaded subjects of the second loaded function series. The results are shown in Tables 7.3 and 7.4. Investigation was concentrated on the four hour period immediately following loading, that is, during the period of maximum BAIBA excretion, and some interesting results were obtained.

It appeared that in certain cases a relatively low urine volume was accompanied by a correspondingly high level of BAIBA excretion. This is perhaps the converse of what might be expected, since normally the lower the urine volume, the greater the concentration, and hence the greater the reabsorption of water and essential metabolites from the kidney tubules. Consider the second

Table 7.4 4 - Thymine Loaded Patients

Comparison of Rate of BAIBA Excretion and Rate of Urine Flow

Sample No.	Sampling Time (hr)	Subject				Mean	
		E.D.C.	A.F.O.	S.J.S.	J.H.R.		
1	24	0.0096	0.0063	0.0044	0.0026	0.0057	A
		0.670	1.440	0.503	0.930	0.886	B
2	2+	0.158	0.117	0.330	0.174	0.195	A
		1.120	1.900	0.475	2.130	1.406	B
3	2	0.363	0.534	1.190	0.444	0.633	A
		1.900	2.030	0.567	2.170	1.667	B
4	4	0.012	0.143	0.188	0.010	0.088	A
		1.050	1.690	0.646	0.550	0.984	B
5	16	0.024	0.0007	0.003	0.008	0.009	A
		1.045	0.750	0.457	1.190	0.860	B
6	2+	0.354	0.244	0.891	0.330	0.454	A
		2.250	0.626	0.408	4.010	1.823	B
7	2	0.172	0.476	1.920	0.199	0.692	A
		1.670	0.725	0.367	1.070	0.958	B
8	4	0.019	0.020	0.412	0.031	0.120	A
		0.770	0.660	0.416	0.449	0.601	B
9	16	0.022	0.002	0.008	0.011	0.011	A
		1.570	0.510	0.407	0.526	0.758	B
10	2+	0.323	0.019	0.317	0.114	0.193	A
		5.170	0.683	0.418	0.742	1.753	B
11	2	0.127	0.171	1.410	0.193	0.475	A
		1.320	0.384	0.483	0.926	0.778	B
12	4	0.015	0.167	0.231	0.011	0.106	A
		1.670	0.675	0.700	2.86	1.476	B
13	16	0.016	0.002	0.0015	0.0045	0.006	A
		0.874	0.761	0.860	0.853	0.837	B
14	2+	0.150	0.299	0.317	-	0.255	A
		1.300	-	0.616	-	0.980	B
15	2	0.265	0.525	1.050	-	0.613	A
		0.926	1.20	0.541	-	0.889	B
16	4	0.016	0.035	0.169	-	0.073	A
		1.020	1.081	0.90	-	1.000	B
17	16	0.013	0.0007	0.002	-	0.005	A
		0.095	1.470	0.847	-	1.074	B

A - BAIBA excretion mg./min.

B - Volume of Urine ml./min.

\+ - Thymine Load (500 mg. orally).

Table 7.3 4 Thymine Loaded Patients - Time Fractionated

Comparison of Urinary BAIBA Output and Related Urine Volume

Sample No.	Sampling Time (hr)	Subjects				Mean	
		E.D.C.	A.F.O.	S.J.S.	J.H.R.		
1	24	19.60	12.80	9.00	5.34	11.68	A
		1360	2940	1050	1895	1811	B
2	2+	19.10	14.00	39.50	20.90	23.37	A
		134	228	57	256	169	B
3	2	44.70	64.00	141.30	53.30	75.82	A
		228	244	68	260	200	B
4	4	2.90	34.40	45.00	2.42	21.18	A
		251	406	155	132	236	B
5	16	22.60	0.64	2.73	7.80	8.94	A
		1005	720	438	1144	827	B
6	2+	42.50	29.20	107.00	39.60	53.08	A
		270	75	49	482	219	B
7	2	20.60	57.30	230.00	24.80	83.17	A
		200	87	44	128	115	B
8	4	4.50	4.77	98.70	7.62	28.90	A
		185	158	100	134	144	B
9	16	21.50	2.30	7.37	10.60	10.49	A
		1505	490	390	505	723	B
10	2+	38.70	2.33	38.20	13.70	23.23	A
		620	82	50	89	210	B
11	2	15.20	20.50	169.50	23.10	57.32	A
		158	46	58	110	93	B
12	4	3.77	40.00	55.30	2.64	25.43	A
		400	162	168	685	354	B
13	16	15.90	1.80	1.49	4.29	5.87	A
		840	730	825	820	809	B
14	2+	18.00	35.90	38.00	-	30.63	A
		156		74	-	115	B
15	2	31.80	63.00	126.00	-	73.60	A
		111	144	65	-	107	B
16	4	3.96	8.32	40.60	-	17.63	A
		244	260	216	-	240	B
17	16	12.80	0.70	2.17	-	5.22	A
		870	1410	804	-	1028	B

\+ - 500 mg thymine administered orally.

X - irradiation exposure between samples 5 and 6.

A - BAIBA mg/sample.

B - Urine volume ml/sample.

series of four thymine-loaded patients (Table 7.3). It was noted before that three of this group showed the same pattern of little or no response to radiation with regard to BAIBA formation. But the fourth subject, namely S.J.S., showed a very marked response with greatly elevated BAIBA levels after loading and even higher still after exposure to X-irradiation. But on studying Table 7.3, it appeared that S.J.S. consistently excreted much smaller quantities of urine that the other three patitents of this group. In fact, at times, S.J.S. had a clearance rate of less than 0.5 ml/min. (Table 7.4).

It is possible then, that this urine volume factor is the reason why S.J.S. showed a different response to the other three subjects. But if this renal factor is the reason for the observed high BAIBA excretion, the mechanism of the system is rather difficult to explain. It is tentatively suggested that there is perhaps a certain critical urinary flow rate, below which the mechanism of renal reabsorption is significantly altered from the normal function. This concept is not new - consider for example the clearance of urea.

Figure 13 shows the curves for the variation of BAIBA excretion with clearance rate, and it seems to indicate that below 0.5 ml/min., the amount of BAIBA

COMPARISON OF BAIBA EXCRETION AND CLEARANCE RATE.

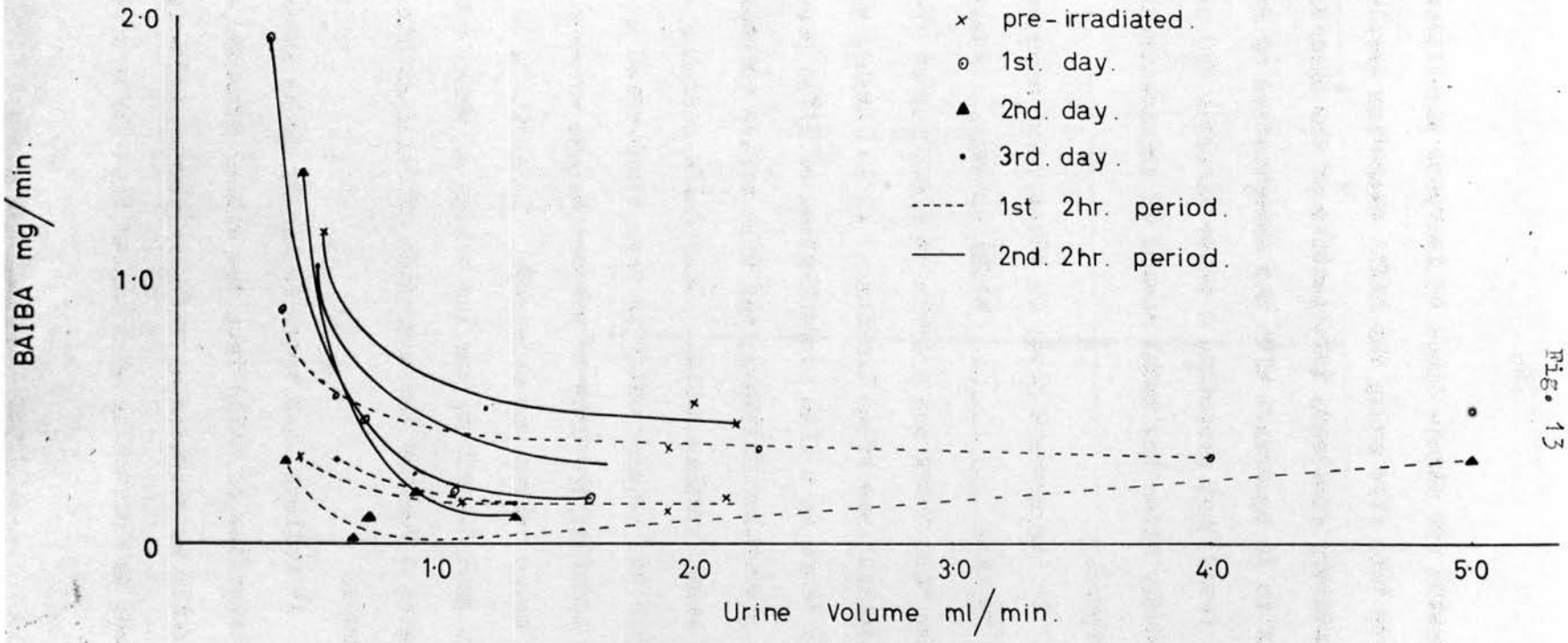


Fig. 13

excreted per minute tends to increase significantly. Above this flow rate, the BAIBA excretion levels remain relatively constant, irrespective of the clearance rate. This is in agreement with the observations of Dent (1947) who found that promoting a water diuresis did not significantly alter the total amount of amino-nitrogen excreted.

In the present study an attempt was made to saturate the Thymine ——→ BAIBA pathway. Table 6.17 indicates that there was a definite elevation of plasma BAIBA concentrations after loading. It is likely, therefore, that there is a high concentration of BAIBA passing into the glomerular filtrate, and thus active reabsorption by the renal tubular cells. That BAIBA escaping metabolism within the tubular cells is then transferred to plasma. But consider the case of subject S.J.S. where a consistently low urine volume was observed. In addition to the mechanism just described, the low volume of urine tends to suggest a further concentration of BAIBA within the tubules.

It follows that there is likely to be excessive reabsorption of BAIBA from the kidney tubules, and a study of Table 6.17 indicates that the plasma BAIBA levels for S.J.S. are generally much higher than those of the other

three subjects in this group. Perhaps in this case, the concentration of BAIBA following loading in the case of a low clearance rate is so great that the active sites of BAIBA reabsorption in the kidney tubules become saturated, and the kidney simply cannot cope with the excess BAIBA which therefore spills over into the urine resulting in relatively high BAIBA levels.

From this it can be concluded that below a certain critical clearance rate in loading function experiments there exists a very real possibility that a renal factor comes into consideration. Therefore in studies of this type it would appear to be essential to ensure an adequate clearance rate and hence it is proposed that sufficient hydration of subjects to be studied is another criterion to be established for the ultimate model for pyrimidine metabolism studies. It is tentatively suggested that a clearance rate equivalent to approximately 0.5 ml/min. is the critical value which must be exceeded.

From a consideration of previous observations, in the final series of two "loaded" patients, two modifications were applied to the original technique.

(i) Administration of thymine orally 2 hours prior to radio-therapy to obviate losses through vomiting.

(ii) Attempt to ensure adequate hydration of

Table 7.5 2 Thymine Loaded Patients

Comparison of Urinary BAIBA Output and Related Urine Volume

Sample No.	Sampling Time (hr.)	Subjects		
		R.J.D.	J.H.	
1	24	9.10	15.00	A
		1063	2305	B
2	2+	7.05	6.96	A
		115	115	B
3	2	26.06	59.60	A
		150	530	B
4	4	10.10	32.40	A
		197	382	B
5	16	4.47	2.68	A
		725	995	B
6	2+	2.34	17.60	A
		76	478	B
7	2	66.50	173.00	A
		78	128	B
8	4	85.30	76.80	A
		144	168	B
9	16	11.90	3.71	A
		425	317	B
10	2+	20.20	8.00	A
		87	28	B
11	2	34.40	52.50	A
		63	40	B
12	4	11.30	36.00	A
		300	118	B
13	16	9.30	4.34	A
		620	710	B

\+ - 500 mg. Thymine administered orally.

X - irradiation exposure between samples 6 and 7.

A - BAIBA mg./sample.

B - Urine volume ml./sample.

Table 7.6 2 Thymine Loaded Patients

Comparison of Rate of BAIBA Excretion and Rate of Urine Flow

Sample No.	Sampling Time (hr.)	Subjects		
		R.J.D.	J.H.	
1	24	0.0045	0.0074	A
		0.521	1.132	B
2	2+	0.059	0.058	A
		0.920	0.920	B
3	2	0.218	0.497	A
		1.25	4.42	B
4	4	0.042	0.135	A
		0.821	2.94	B
5	16	0.0046	0.0028	A
		0.755	1.037	B
6	2+	0.0195	0.147	A
		0.634	3.980	B
7	2	0.554	1.440	A
		0.650	1.067	B
8	4	0.356	0.320	A
		0.600	0.700	B
9	16	0.0124	0.0038	A
		0.443	0.330	B
10	2+	0.169	0.067	A
		0.725	0.234	B
11	2	0.287	0.438	A
		0.525	0.334	B
12	4	0.047	0.150	A
		1.25	0.492	B
13	16	0.0097	0.0045	A
		0.646	0.740	B

\+ - 500 mg. Thymine administered orally.

X - Irradiation exposure between samples 6 and 7.

A - BAIBA mg./min.

B - Volume of Urine ml./min.

subjects.

As regards the latter, with a few exceptions the clearance rates were generally maintained above the established limit of 0.5 ml/min. (Tables 7.5 and 7.6). The two subjects R.J.D. and J.H. showed quite a similar marked response with respect to elevated urinary BAIBA levels, indicating the possible success in overcoming the two aforementioned variable factors.

8. SOME GENERAL OBSERVATIONS

8.1 Valine Studies

During the course of investigation into a suitable model for BAIBA metabolism studies, a number of interesting observations were made. The first of these involved valine studies. Mention has already been made of Coon's proposed pathway for valine to BAIBA metabolism (Figure 8) and he would appear to have well established its existence in the pig (Coon 1955, Robinson and Coon 1957, Kupiecki and Coon 1957). Therefore it was thought to be of interest to see if the results of the investigation into BAIBA and valine metabolism indicated any evidence of this pathway occurring in man. For, if this was so, valine might well prove to be at least a subsidiary source of the increased BAIBA levels commonly found in the urine of humans, subsequent to exposure to ionising radiation.

Preliminary observations indicated an elevation in both urinary BAIBA and valine in humans after X-irradiation treatment. The following table gives the mean values for approximately twenty subjects who were not loaded with thymine.

Table 8.1 Urinary BAIBA and Valine

	Pre-Irrad.	Day 1	Day 2	Day 3	Day 4
BAIBA (μM/24 hr.)	121	155	207	166	174
Valine (μM/24 hr.)	57	83	85	72	57
Ratio BAIBA/Valine	2.13	1.87	2.44	2.31	3.05

With regard to the possible inter-relationship between BAIBA and valine metabolism, there are three main alternatives.

(i) BAIBA and valine metabolism are completely independent of each other and the observed increases are basically a manifestation of general tissue damage arising from exposure to ionising radiation and bear no relationship to each other.

(ii) Increased urinary valine appears as a result of increased production of BAIBA.

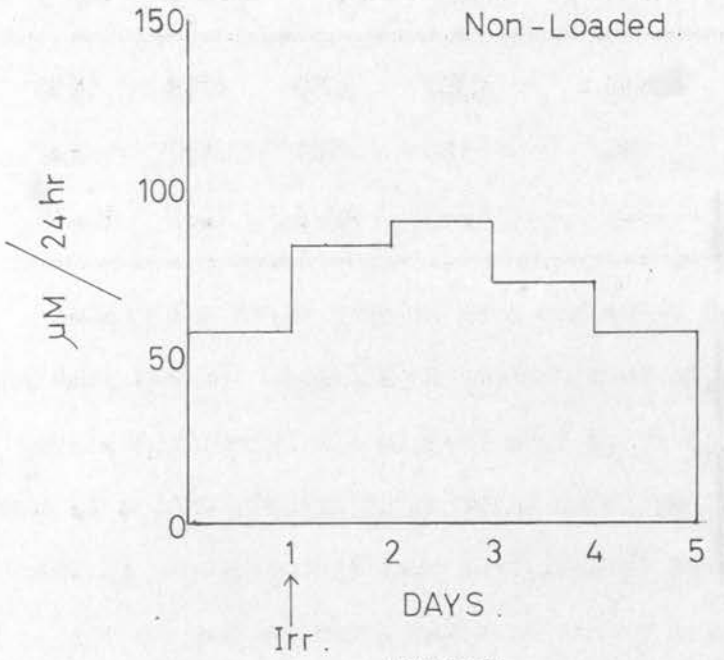
(iii) Increased urinary BAIBA appears as a result of increased production of valine.

Table 8.1 shows a fairly constant ratio of BAIBA: Valine values which might indicate a possible metabolic relationship between the two. It appeared to be worthwhile following up this observation in the "thymine-loaded" experiments.

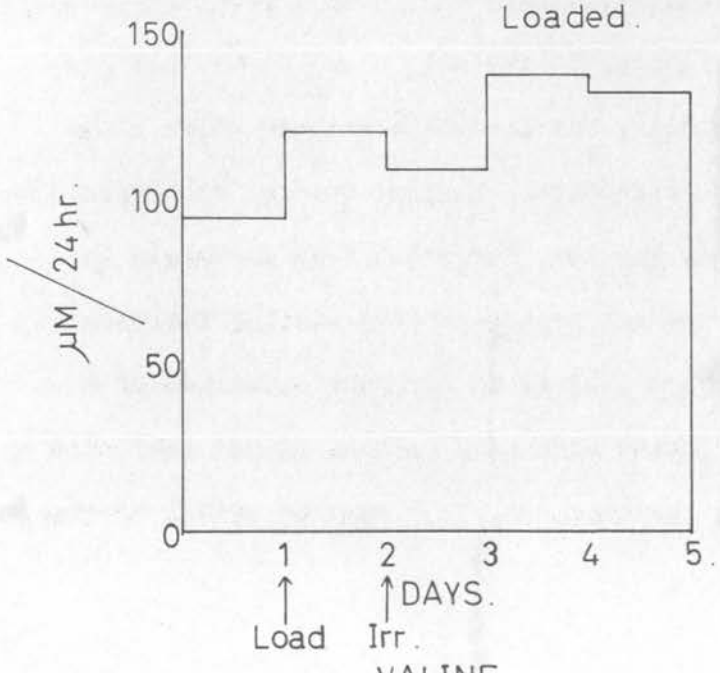
Fig. 14

URINARY VALINE.

Mean Values.



VALINE.



VALINE.

Table 8.2 Urinary BAIBA and Valine (Thymine Loaded Subjects)

	Pre-Irrad.	Load	Day 1	Day 2	Day 3
BAIBA (μM/24 hr.)	611	2307	3086	1799	1630
Valine (μM/24 hr.)	94	120	109	137	132
Ratio BAIBA/Valine	6.5	19.3	28.3	13.3	12.35

Table 8.2 shows the mean urinary BAIBA and valine levels for seven thymine-loaded patients. A 4-5 fold increase in BAIBA after both loading and X-irradiation was obvious. But while an increase in urinary valine is also apparent, Figure 14 indicates that the magnitude of the increased valine excretion after X-irradiation is the same in both "loaded" and "non-loaded" groups of subjects (approximately 45% increase in each case). It follows that these results indicate that Coon's proposed pathway, if it exists in man, is probably a non-reversible one, since on saturating the metabolic pathway under study with BAIBA, as a result of thymine loading and X-irradiation treatment, the very large 4-5 fold increases in BAIBA levels are not accompanied by similar increases in urinary valine. That is to say, the existence of a BAIBA ⟶ Valine metabolic pathway is not indicated by the foregoing observations. It must be noted, however,

Table 8.3 Urinary Valine (μM/24 hr.)

Thymine Loaded Subjects

Subject	Pre	Thymine Load	Day 1	Day 2	Day 3
I.McD.	84	30	54	87	135
O.M.H.	163	224	147	200	175
T.G.W.	53	67	121	67	130
G.S.H.	90	90	111	135	-
D.W.	88	69	72	200	73
D.Mc.K.	125	272	155	141	-
E.D.C.	57	89	102	132	146
Mean	94	120	109	137	132
Mean % Change	-	+ 28%	+ 16%	+ 46%	+ 40%
High	163	272	155	200	175
Low	53	30	54	67	73
No. of Observations	7	7	7	7	5

Subjects given 500 mg Thymine orally.

Therapeutic dose - 150 rad. partial-body in abdominal region.

that Table 8.2 indicates an increase in valine merely after thymine loading but further investigation produced a possible explanation for this observation.

Table 8.3 shows the urinary valine levels for the seven "loaded" subjects. Most of them initially excreted valine within the normal range. That is, less than 10 mg/24 hr. (90 μM/24 hr.) as quoted in the literature (Evered, 1956). Two of them appeared to normally excrete elevated amounts of valine (15-20 mg/24 hr.). To determine if this had any particular significance, the group was divided into what might tentatively be termed "normal" and "high" valine excretors. The comparative results are shown in the following table:-

Table 8.4 Urinary valine (μM/24 hr.)

	Pre-Irrad.	Load	Day 1	Day 2	Day 3
Normal Valine	74	69	92	124	121
High Valine	144	248	151	170	175
Ratio High/Normal	1.96	3.60	1.64	1.37	1.45

The normal valine excretors exhibited the expected pattern. There was no significant change in urinary valine after thymine loading (disproves BAIBA ⟶ Valine pathway), but a significant increase in this

urinary metabolite after X-irradiation exposure (+ 67%) in agreement with the observations on the "non-loaded" subjects. In the case of the two "high valine" excretors, the response was quite different. There was a very significant increase in urinary valine after loading (+ 75%) which could account for the apparent increase after loading when the group was considered as a whole (Table 8.2).

On irradiation, the urinary valine levels in this latter group dropped below the control loaded level, the opposite response to that of the normal valine excretors.

These observations are difficult to interpret, but it seems indicative that there is a possibility that a reverse pathway of Coon's proposed metabolic system, that is BAIBA ——→ Valine, does exist in those humans which exhibit a tendency to high normal urinary valine levels. If this is so then the Valine ——→ BAIBA system via methyl malonic acid semialdehyde might well exist in these cases. In working with thymine-loaded subjects the approach has, in effect, been from the opposite end of the possible pathway, from Coon's investigations. The existence of this pathway can only be fully established by valine-loading experiments, and it is intended to carry out some investigations to this effect in the future,

loading both human subjects and animals with valine in an attempt to saturate the metabolic system in question and at the same time to study the effect of X-irradiation on valine metabolism. The significance of the apparent drop in urinary valine below control loaded levels in the "high" valine excretors after X-irradiation might be as a result of the stimulation of the possible Valine ——→ BAIBA pathway.

Obviously more work must be done on the study of valine metabolism, and all that can be stated at present is as follows:-

(i) It is possible that there exists some apparently normal humans who exhibit a tendency to "high" valine excretion.

(ii) It is possible that the difference between "high" and normal valine excretors is a renal factor. Table 6.3, indicates that there is no significant difference in plasma levels between the two types.

(iii) In "normal valine" subjects there is no indication of the BAIBA ⟸ Valine system.

(iv) In "high valine" subjects there is a possibility that the BAIBA ——→ Valine system exists.

8.2 Comparison of High and Low BAIBA Excretors

Gartler (1959) in his investigations on the biochemical

Table 8.5 Comparison of Urinary BAIBA in High and Low Excretors

Effect of Thymine Loading

SUBJECT	URINARY BAIBA (mg/24.hr.)			
	PRE	Increase above Pre after loading	Increase above Pre 1st day post-irradiation	Increase above Loaded level, 1st day post-irradiation
LOW EXCRETORS				
I.Mc.D.	7.2	46.5	188.5	141.0
O.M.H.	25.0	66.2	80.3	14.1
T.G.W.	15.7	75.5	140.8	65.5
G.S.H.	46.5	27.5	272.5	245.0
E.D.C.	19.6	49.7	69.5	19.8
A.F.O.	12.8	100.2	81.2	- 19.0
S.J.S.	9.0	219.5	434.4	214.8
J.H.R.	5.3	79.1	77.3	- 1.8
R.J.D.	9.1	38.6	156.9	118.4
J.H.	15.0	86.6	256.1	169.5
HIGH EXCRETORS				
D.W.	96.7	653.3	509.3	- 144.0
D.Mc.K.	217.0	268.0	473.0	205.0

nature of the underlying genetic mechanism in β-aminoisobutyric aciduria, reported relatively small and inconsistent differences in BAIBA excretion between low and high excretors after the oral administration of thymine. In contradiction to this, the results obtained in this series of investigations seemed to indicate a significant difference (Table 8.5). With the possible exception of subject S.J.S., an old man of about seventy, the low excretors tended to show a much smaller increase in urinary BAIBA than did the high excretors, after the oral administration of 500 mg thymine in each case. Thus perhaps oral loading with a suitable precursor tends to magnify the difference in BAIBA metabolism in low and high excretors. There are four possible factors which could account for this difference:-

(i) Lack of enzyme system for BAIBA breakdown in high excretors.

(ii) Renal defect.

(iii) Difference in precursor metabolism - i.e. a difference in thymine utilization.

(iv) Block in precursor metabolism.

Enzyme Hypothesis:-

The main pathway of BAIBA catabolism is considered to be as follows:-

Table 8.6 3 High Excretors

Urinary β-Aminoisobutyric Acid (μM/24 hr)

Subject	Pre	1	2	3	4
R.S.	2290	2470	940	1660	2160
M.M.	3910	4000	3110	2850	1976
J.R.N.	576	866	595	394	-
Mean	2359	2445	1548	1635	2068
Mean % Change	-	+ 3.5%	- 34.4%	- 32.1%	- 12.9%

Individual Mean % Changes

Subject	Pre	1	2	3	4
R.S.	-	+ 7.3 %	-59.0 %	- 27.5%	- 5.67%
M.M.	-	+ 2.25%	-20.5 %	- 27.1%	-49.5 %
J.R.N.	-	+33.5 %	+ 3.19%	- 31.6%	-

Plasma β-Aminoisobutyric Acid (μM/100 ml)

Subject	Pre	1	2	3	4
R.S.	0.29	0.73	1.02	1.96	1.75
M.M.	0.61	0.82	0.68	0.74	-
J.R.N.	0.73	1.23	-	-	-

Table 8.7 3 Low Excretors

Urinary β-Aminoisobutyric Acid (μM/24 hr)

Subject	Pre	1	2	3
J. Mc.	76	117	146	175
W.T.	114	125	414	166
J.S.	129	50	205	143
Mean	106	97	255	161
Mean % Change	-	- 8.5%	+ 141%	+ 52%

Individual Mean % Changes

Subject	Pre	1	2	3
J. Mc.	-	+ 54%	+ 92%	+ 130%
W.T.	-	+ 10%	+ 263%	+ 46%
J.S.	-	- 158%	+ 59%	+ 11%

Plasma β-Aminoisobutyric Acid (μM/100 ml)

Subject	Pre	1	2	3
J. Mc.	0.28	0.42	0.19	0.26
W.T.	1.50	1.48	1.22	0.50
J.S.	1.37	1.31	1.25	1.00

BAIBA

↕ Transaminase (Vit.B6) Cofactor

Methylmalonic Acid ⟶ Succinic Acid $\xrightarrow[\text{Cycle}]{\text{KREBS}}$ CO_2.

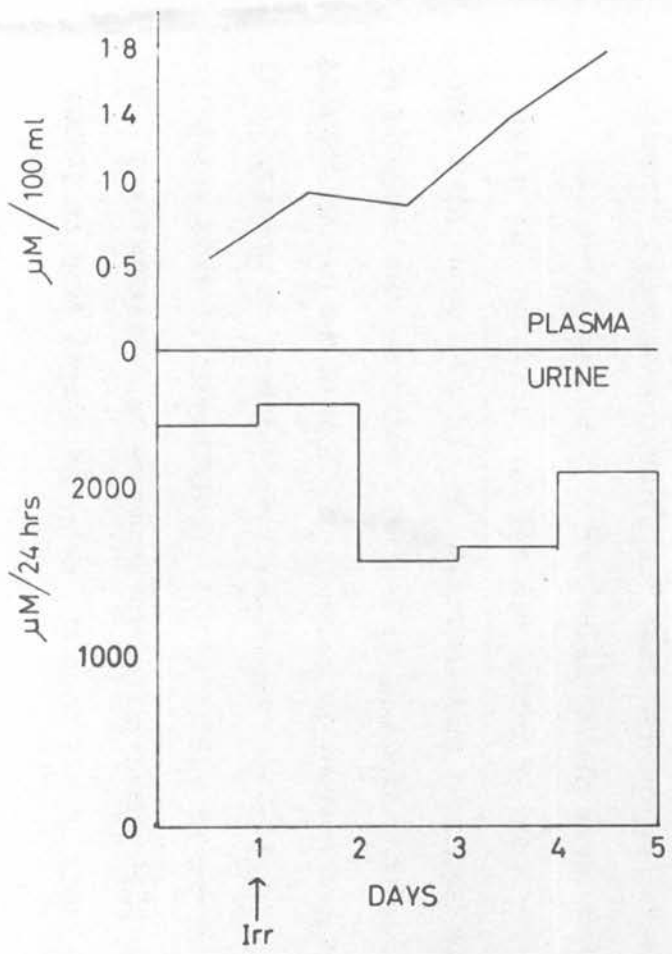
Semialdehyde

It is generally recognised that BAIBA is converted to methylmalonic acid semialdehyde by a pyridoxal phosphate (Vitamin B6) dependent transaminase (Armstrong et al., 1963). It is thought that this transaminase is normally absent or inhibited in "high" BAIBA excretors. On exposure to ionising radiation this enzyme is thought to be inhibited in normal BAIBA excretors whence it follows that they exhibit the observed elevated levels of urinary BAIBA (Bates, Smith and Smith, 1964). Tables 8.6 and 8.7 compare the urinary and plasma BAIBA levels for three "low" and three "high" excretors. They were all "non-loaded" subjects. After X-irradiation the following interesting pattern of response was apparent:-

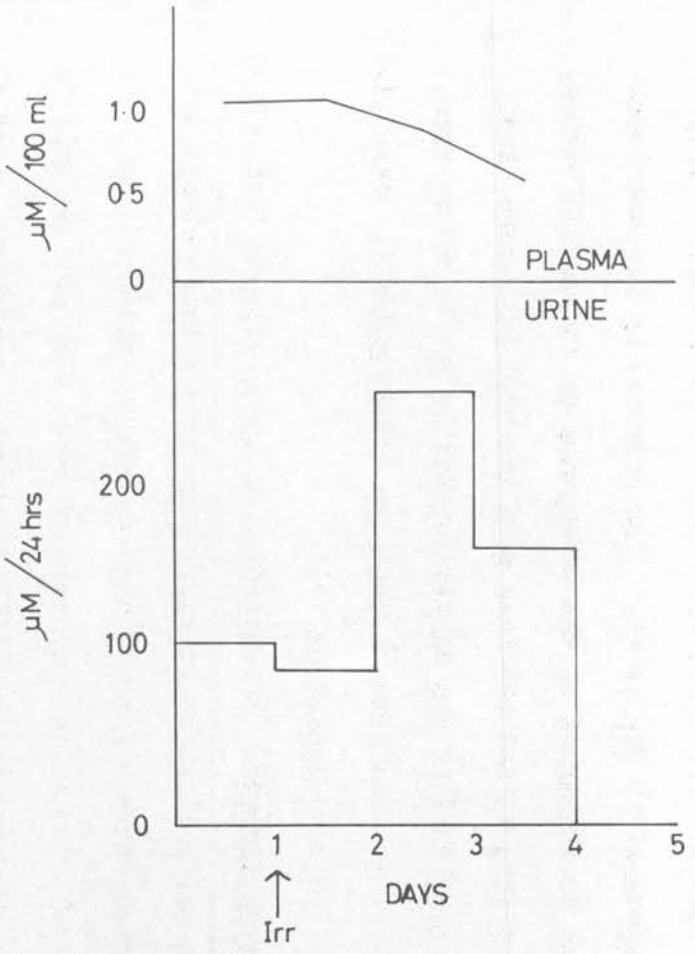
LOW EXCRETORS	HIGH EXCRETORS
Urine BAIBA rises	Urine BAIBA drops
Plasma BAIBA drops slightly	Plasma BAIBA rises

In other words, the two groups appear to show a

BAIBA LEVELS IN LOW AND HIGH EXCRETORS FOLLOWING X-IRRADIATION (150 Rads)



3 High Excretors



3 Low Excretors

Fig. 15

completely different response. This is more clearly expressed in Figure 15. The response by low excretors can be explained on the assumption of enzyme inhibition. But this is not the case with the high excretors, indicating that if the enzyme inhibition hypothesis is true, there is some other subsidiary effect which is stimulated by ionising radiation.

Renal Defect:- A comparison of urine volumes (Table 7.1) shows no significant difference in the daily output of urine between low and high excretors indicating that merely the volume and rate of urine flow are not related to elevated urinary BAIBA in certain subjects. However, it is possible that "high" excretors have a reduced ability to reabsorb BAIBA, and if this was the case, it is to be expected that "high" excretors would normally show lower plasma BAIBA levels than low excretors. A comparison of tables 8.6 and 8.7 indicates only a fair agreement to this extent. It might be postulated, then, that upon exposure to ionising radiation the capacity of high excretors to reabsorb BAIBA from the kidney tubules is increased. Hence the observed drop in urinary BAIBA and the elevation of the plasma levels of this metabolite in high excretors after exposure to X-irradiation. But if this is so, then the observed slight drop in plasma

BAIBA levels in low excretors after exposure to X-irradiation can only be explained on the assumption that the mechanism of BAIBA tubular reabsorption in low and in high excretors is quite different.

Block in Precursor Metabolism:- It is possible that in low excretors there is some block in the thymine ⟶ BAIBA pathway, and further that this block is to some extent released after exposure to ionising radiation. In order to establish this theory it is necessary to follow each of the intermediary metabolites in the thymine degradation pathway.

Thymine ⟷ Dihydrothymine ⟷ β-Ureidoisobutyric Acid.

↓

BAIBA

It is intended to develop suitable continuous flow analytical techniques for the rapid determination of these metabolites. However, in view of the fact that the plasma BAIBA levels in low and high excretors are somewhat similar (Tables 8.6/8.7) it would appear to be most unlikely that a block in the thymine ⟶ BAIBA pathway exists in the former group.

Differential Thymine Utilization

Table 8.5 indicated that the high excretors tended

to utilize thymine more readily for BAIBA formation than the low excretors. This was confirmed in the plasma studies. The following table compares the plasma BAIBA levels in both high and low excretors after loading and exposure to ionising radiation.

Table 8.8 Plasma BAIBA levels (μM/100 ml.)

	C	C+3	C+24	X+3	X+12	X+24	X+27	X+48	X+51
High Excretors	1.04	4.41	0.70	4.34	0.47	0.63	3.96	0.81	2.30
Low Excretors	1.11	1.69	1.14	2.00	0.94	0.81	1.94	2.02	1.38
Ratio High/Low	0.95	2.61	0.61	2.17	0.50	0.78	2.02	0.41	1.67

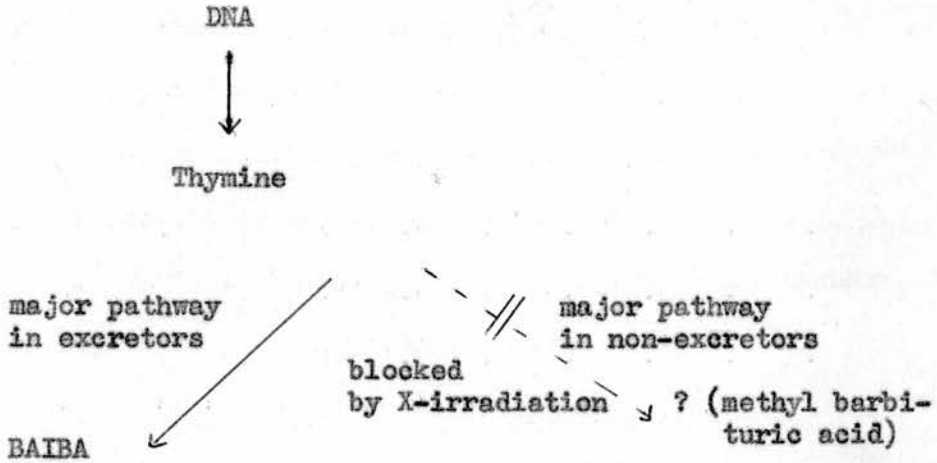
C - Control — 500 mg thymine orally immediately

X - Irradiated — after C, X, X+24, X+48.

Three hours after thymine loading the plasma BAIBA levels in the high excretors were double that of the low excretors. This indicates a possible differential utilization of thymine between the two groups although it appears that at normal control levels there was no significant difference in plasma BAIBA levels between low and high excretors.

The possibility of an alternative pathway for thymine breakdown, while on the evidence of Fink's work highly improbable, makes interesting speculation.

Considering the fact that only some 10% of the population excrete BAIBA in appreciable quantities, this might indicate that there is another pathway of thymine degradation present in non-excretors, and that the BAIBA pathway is the abnormal one.



One possibility for this alternative pathway is the oxidative degradation of thymine to methyl barbituric acid. This metabolic reaction has been demonstrated in certain micro-organisms (Hayaishi and Kornberg, 1952). The methyl barbituric acid might then be hydrolysed to urea and methyl malonic acid. Thymine oxidation ⟶ 5-Methyl Barbituric Acid ⟶ Urea + Methyl Malonic Acid.

It is possible in the case of low excretors, that on exposure to X-irradiation some enzyme system on the alternative pathway is partially inhibited, thus

Table 8.9 3 Low Excretors

Urinary Valine (μM/24 hr)

Subject	Pre	Day 1	Day 2	Day 3	Day 4
J. Mc.	66	115	36	48	-
W.T.	26	69	74	52	-
J.S.	83	180	75	142	76
Mean	58	121	62	81	76
Mean % Change	-	+ 109%	+ 7%	+ 40%	+ 31%
No. of Obs.	3	3	3	3	1

Plasma Valine (μM/100 ml plasma)

Subject	Pre	Day 1	Day 2	Day 3	Day 4
J. Mc.	25.2	23.2	25.3	27.6	-
W.T.	28.2	23.6	33.4	32.5	-
J.S.	37.8	21.1	33.4	45.0	40.6
Mean	30.4	22.6	30.7	35.0	40.6
Mean % Change	-	- 35%	+ 1%	+ 13.5%	+ 33.5%
No. of Obs.	3	3	3	3	1

promoting the BAIBA pathway with the subsequent elevation of BAIBA in the urine.

Certainly, the results obtained in this series of studies indicate some form of differential thymine utilization between low and high excretors in agreement with the observations of Gartler (1959). However, as the control plasma BAIBA levels are more or less similar in both groups, it follows that the ability to produce BAIBA is apparently the same in low and high excretors.

Therefore, although the observed responses to thymine loading and X-irradiation are difficult to explain, it seems most likely that the occurrence of high BAIBA excretion in otherwise apparently normal individuals results from one of the following two factors, or perhaps a combination of both:-

(i) Decreased tubular reabsorption.

(ii) Decreased ability to metabolise BAIBA - i.e. enzyme blockage.

Valine metabolism in high excretors

In view of the possible metabolic relationship between valine and BAIBA (Coon 1955, 1957), comparison of urinary and plasma valine levels in low and high excretors was made. (Tables 8.9 and 8.10). These were non-loaded subjects. The pattern of response in both groups

Table 8.10 3 High Excretors

Urinary Valine (μM/24 hr)

Subject	Pre	Day 1	Day 2	Day 3	Day 4
R.S.	44	120	25	85	27
M.M.	109	152	188	136	82
J.R.N.	64	88	43	35	-
Mean	72	120	85	85	36
Mean % Change	-	+ 67%	+ 18%	+ 18%	- 50%
No. of Obs.	3	3	3	3	2

Plasma Valine (μM/100 ml plasma)

Subject	Pre	Day 1	Day 2	Day 3	Day 4
R.S.	26.9	21.7	33.0	34.4	30.2
M.M.	39.2	34.9	45.0	38.8	-
J.R.N.	38.8	35.7	-	-	-
Mean	35.0	30.4	39.0	36.6	30.2
Mean % Change	-	- 15%	+ 11.5%	+ 4.5%	- 16%
No. of Obs.	3	3	2	2	1

was the same, with a significant post-irradiation increase in urinary valine on Day 1 accompanied initially by an apparent moderate decrease in plasma valine. This might indicate decreased renal reabsorption after exposure to ionising radiation. It is significant that there appears to be no difference in valine metabolism between low and high excretors. It follows therefore, that high excretors do not exhibit any apparent abnormality in valine metabolism. Therefore it is most unlikely that valine contributes to the source of the elevated urinary BAIBA found in high excretors.

8.3 Possible effect of Thymine Loading on Urinary amino acids

During the latter studies involving BAIBA metabolism, thymine was administered orally to the subjects in an attempt to saturate the specific pyrimidine metabolic pathway in order to facilitate the differentiation between physiological and pathological fluctuation of BAIBA after exposure to ionising radiation. In effect, it is possible that these loading function tests create abnormal metabolic conditions, apart from the obvious BAIBA elevation, a factor which is perhaps disadvantageous if the findings of this thesis are to be applied to human dosimetry, and the ultimate adoption of some biochemical indicator of X-irradiation exposure. It was therefore decided to

investigate the possibility that "thymine-loading" might affect the urinary levels of the metabolites, other than BAIBA, which were studied in this thesis.

The following table compares "non-loaded" and "loaded" urinary levels for eight metabolites, and it was apparent that "thymine-loading" might well have an effect on the metabolism of some urinary amino acids.

Table 8.11 Effect of "thymine-loading" on urinary amino acids

Amino Acid	Mean Pre-Irradiation Values (μM/24 hr.)		Mean % Change
	Non-Loaded	Loaded	
Taurine	751	1048	+ 40%
Cystine	69	111	+ 61%
Serine	463	731	+ 58%
Ethanolamine	462	476	+ 3%
Glutamic Acid	46	75	+ 63%
Cysteic Acid	111	119	+ 7%
Valine	89	95	+ 7%
BAIBA	178	1008	+ 466%

Study on 6 loaded subjects.

(BAIBA - 8 subjects, excluding high excretors).

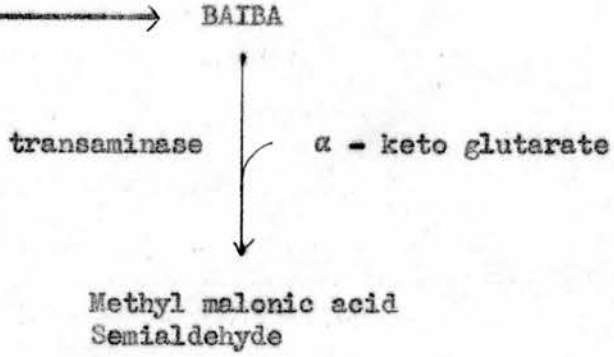
Apart from the natural BAIBA elevation (approximately 450% increase), four other metabolites showed

an apparent increase. These were Taurine, Cystine, Serine and Glutamic Acid. This observation is rather surprising, particularly as the increases were fairly substantial. It is unlikely that there can be a direct metabolic relationship between thymine and such sulphur-containing metabolites as taurine and cystine. A more probable theory is that there is some differential reabsorption effect in the kidney tubules. It has been reported that intraperitoneal administration of amino acids to the mouse resulted in an increased urinary output of taurine (Gilbert et al 1960). In particular, administration of β-amino acids produced the highest concentrations of urinary taurine. Thymine being an important precursor of BAIBA, the subjects in this study have, in effect, been "loaded" with a β-amino acid (i.e. BAIBA). Gilbert (1960) has postulated that β-amino acids and taurine are reabsorbed at the same sites on the kidney tubules, and that administration of β-amino acids competitively displaces taurine from its usual reabsorption sites with the subsequent elevation of urinary taurine.

It is possible then, that as a consequence of "thymine-loading" and subsequent high concentration of BAIBA, taurine is elevated in the urine as a result of

this renal reabsorption factor. At the same time, it is possible that amino acids such as glutamic acid, serine and cystine are competitively displaced in the same way. Certainly none of these amino acids are essential ones. But there might also be some significance in the fact that taurine, serine and cystine are all involved in the same metabolic pathway.

Urinary glutamic acid might well be elevated as a result of a direct metabolic connection with thymine.



Glutamic acid is a "by-product" of the combination of BAIBA and α-keto glutarate to form methyl malonic acid semialdehyde. Therefore it is possible that if a high concentration of BAIBA (as a result of thymine loading) stimulates this transaminase reaction, then increased formation of glutamic acid will result. But if this is the case, then a further increase in glutamic acid might

be expected after exposure to ionising radiation. There was no indication of this increase in these experiments (Table 6.2). However, the work of Kupiecki and Coon (1957) indicated that the BAIBA transaminase reaction is in fact a second order reaction, and as such will be dependent on the BAIBA concentration. Therefore increased BAIBA concentration should promote this transaminase reaction and subsequent glutamic acid formation.

It follows that "thymine-loading" may well upset the metabolic balance of other apparently unrelated metabolites and therefore the translation of the results of these function tests to normal metabolic systems must be done with caution.

9. SUMMARY AND CONCLUSIONS

9.1 Summary

(i) Urine and plasma samples have been collected from in-patients receiving radio-therapy in an attempt to develop a suitable biochemical indicator of radiation damage in the human. These subjects received treatment on a prophylatic basis and therefore closely approximated to normal healthy males.

(ii) Amino acid metabolism, is allegedly changed following exposure to ionising radiation. To confirm this some eight amino acids including the sulphur-containing cysteic acid and cystine and also a related derivative, taurine, have been investigated in man following exposure to low-level partial-body X-irradiation. Only two metabolites indicated a significant response at the low levels of exposure used. These were taurine and BAIBA.

(iii) Daily urinary taurine excretion is variable, being dependent upon dietary intake. This factor makes taurine somewhat unsuitable as a biological indicator. Therefore emphasis in this thesis has been concentrated on BAIBA, a breakdown product of nucleic acid. The level of this metabolite in individuals is relatively constant under normal conditions.

(iv) Because of difficulties in the analysis of BAIBA, a rapid technique involving ion-exchange column chromatography and continuous flow colorimetry has been developed. This was necessary in order to fulfil the programme which required multiple analysis.

(v) A suitable experimental model for the study of the effect of ionising radiation on the pyrimidine metabolic pathway has been postulated

viz. $\text{DNA} \rightleftharpoons \text{Thymine} \xrightarrow{1} \text{BAIBA} \xrightarrow{2} CO_2$

In the above simplified scheme of the pyrimidine pathway, the enzyme system involved in Reaction 1 is fast relative to the enzyme system involved in Reaction 2. However in the normal individual (excluding high excretors) there is no accumulation of BAIBA in body fluids because the rate of formation of BAIBA is such that the enzymes in Reaction 2 can cope with the amount of BAIBA formed. It is only under pathological conditions for example, exposure to high level of radiation, that excessive catabolism of thymine occurs. Under these circumstances, BAIBA accumulates in the plasma and is excreted by the kidneys, because Reaction 2 becomes saturated. In order to artificially saturate the BAIBA pathway and hence stress the enzyme system, thymine has been administered to

individuals before and after irradiation.

(vi) The possibility of renal effects following exposure to ionising radiation has been considered.

(vii) The possible metabolic relationship in man between valine and BAIBA has been studied.

(viii) Comparison of the effect of ionising radiation on normal and genetically "high" BAIBA excretors has been made.

9.2 Conclusions

(i) Increased urinary excretion of taurine and BAIBA has been observed in patients exposed to partial body X-irradiation.

(ii) There is no indication of a dose-dependent response in either case; that is, there is no direct quantitative relationship between the irradiation level and the degree of amino aciduria.

(iii) It is possible that hypertaurinuria following low-level exposure to ionising radiation results from the release of taurine from a pre-formed source in the lymphocytes which are exceptionally radio-sensitive. There may also be a supplementary decreased renal reabsorption effect in the kidney tubules. Plasma taurine is not significantly elevated after X-irradiation exposure at low dose levels.

(iv) The observed increase in urinary BAIBA following exposure to ionising radiation is possibly the result of a combination of three factors:-

(i) Decreased nucleotide phosphorylation.

(ii) Increased DNA catabolism.

(iii) BAIBA transaminase blockage.

Factors (i) and (ii) both result in more thymine being available for BAIBA formation.

(v) The levels of partial-body exposure utilized in this study are probably too low to be of major biochemical significance.

(vi) Biochemical lesions resulting from these low doses appear to be short term effects, the metabolic systems returning to apparently normal function in about four days or less. This infers that normal physiological homeostatic mechanisms which function in the re-establishment of the amino acid balance are not significantly affected by low-level exposure to ionising radiations, and repair processes continue to function. Therefore the differentiation between physiological and pathological fluctuations at low levels of exposure is extremely difficult.

(vii) In view of the fact that the nutritional condition and various forms of disease have a

influence on blood and urine levels of amino acids - an influence which may be greater than that of irradiation - it is perhaps unlikely that amino acids under non-loading conditions are the answer to a biochemical indicator of low-level radiation exposure.

(viii) The loading function test when fully developed is considered to be a suitable method for demonstrating sub-clinical responses to ionising radiation. In particular, thymine function tests are proving useful in a study of the related pyrimidine metabolic pathway.

(ix) The function test shows that thymine is undoubtedly a major precursor of BAIBA in man.

(x) The wide variation in individual responses to ionising radiation may result from differential reabsorption effects in the kidney tubules brought about by X-irradiation. There is a distinct possibility that Taurine and BAIBA are reabsorbed at the sames sites on the kidney tubules and that in the event of excessive production of BAIBA, as a result of thymine loading or X-irradiation exposure, taurine may be competitively displaced from its usual sites of reabsorption and consequently elevated in the urine. Other amino acids, for example serine

and cystine, may be affected in the same way.

(xi) There is no obvious relationship between BAIBA and Valine metabolism. The results obtained give no indication that Coon's proposed metabolic pathway (Valine ——→ BAIBA) exists in man.

(xii) It is possible that "low" and "high" BAIBA excretors react in a fundamentally different way to X-irradiation exposure.

10. RECOMMENDED STUDIES INVOLVING THYMINE LOADING

While the results of this thesis are rather disappointing from the point of view of biochemical dosimetry, it is intended to continue with studies relating thymine-loading and radiation effects. Quite apart from the well-established radiosensitivity of DNA, the pyrimidine metabolic pathway is also important in the study of neoplastic disease and worthy of further investigation. With regard to urine and blood sampling from human subjects and the study of the metabolites therein after "loading" and radiation exposure, the following points are to be noted:-

(i) Since the biochemical lesions resulting from low-level exposure are relatively short-term effects, sampling of urine should be carried out during the few hours immediately post-irradiation. Pooled 24 hour urine samples are perhaps of little specific value, particularly in the case of loading function tests applied to irradiation studies when the most dramatic changes occur in the first 2 or 4 hours following "loading". In particular blood sampling should also be carried out as frequently as is possible. With the exception of BAIBA, small changes in urinary metabolites may be of little significance when one considers the normal daily variations, but small changes in blood metabolites may be of the greatest importance in the

detection of radiation damage. It is possible that from the dosimetry point of view, in the case of low-level exposure the answer lies perhaps in the blood metabolite levels.

(ii) The vomiting reaction of most humans to X-irradiation must be carefully considered and "loading" function tests planned in such a way that there is no loss of the orally administered substance through vomiting. It is hoped, if possible, to administer the thymine and eventually L- BAIBA to subjects by intravenous injection.

(iii) The subjects' environmental conditions should be kept as similar as possible. Metabolic ward conditions are essential. The subjects will be hydrated throughout the treatment to ensure adequate urine flow and hence minimise variations between individuals resulting from renal factors.

(iv) In function tests it is probable that complete saturation of the particular metabolic pathway is necessary before it is possible to differentiate between physiological and pathological changes. It may therefore be necessary to further increase the oral dose of thymine in these studies.

11. FUTURE PROJECTS

In conjunction with the programme described it is planned to carry out a number of other related studies including the following:-

11.1 Related to Thymine Metabolism

(i) Thymine Studies

To measure the thymine levels in blood and urine in both thymine-loaded and non-loaded subjects receiving radiotherapy. A rapid method for thymine determination has been developed, involving ion-exchange absorption column chromatography and continuous flow ultra-violet spectroscopy. This method will also be suitable for thymidine determination. Preliminary studies indicate that thymine is rapidly metabolised to BAIBA in man, rabbit, rat and mouse (Smith, Chapman and Sturrock. Unpublished 1967).

(ii) DNA Studies

This will involve a modified version of the Dische reaction (Burton, 1956) incorporated into an automated analytical system. Eventually it is hoped to be possible to carry out a complete "monitoring" of the DNA pyrimidine metabolic pathway, in order that the reaction of this system to ionising radiation can be closely followed.

(iii) Carbon-14 Studies

It is planned to carry out further studies with C^{14} labelled thymine and BAIBA in order to follow the kinetics of the pyrimidine system and the way(s) in which the thymine and BAIBA are utilised.

(iv) The Orotic Acid Cycle

It is hoped to study the Orotic acid cycle and investigate the possibility of a "feed-back" system for DNA restoration after radiation damage via this cycle.

(v) Enzyme Inhibition by methods other than X-irradiation

It is planned to use various suitable chemical agents to block the pyrimidine metabolic system and related pathways at various selected points. For example, it is intended to block succinate dehydrogenase (thus blocking the Krebs Cycle) by administration of excess malonic acid which competitively inhibits the enzyme (Thorn, 1953). After irradiation this may result in a biochemical lesion, prior to Krebs Cycle, becoming more readily demonstrable. Thus it might lead to a greater accumulation of BAIBA, or perhaps stimulate another metabolic pathway for BAIBA.

(vi) High/Low BAIBA Excretor Studies

This of course will be strictly limited by the number of high excretors available. It would appear that on average 1 in 10 patients investigated are high excretors. This means that to obtain any information of statistical significance on "high excretors" will involve perhaps 200 patients. However, as these "high excretors" provide a unique opportunity for the study of BAIBA metabolism it is planned to continue a comparison of the response of "low" and "high" BAIBA excretors to ionising radiations.

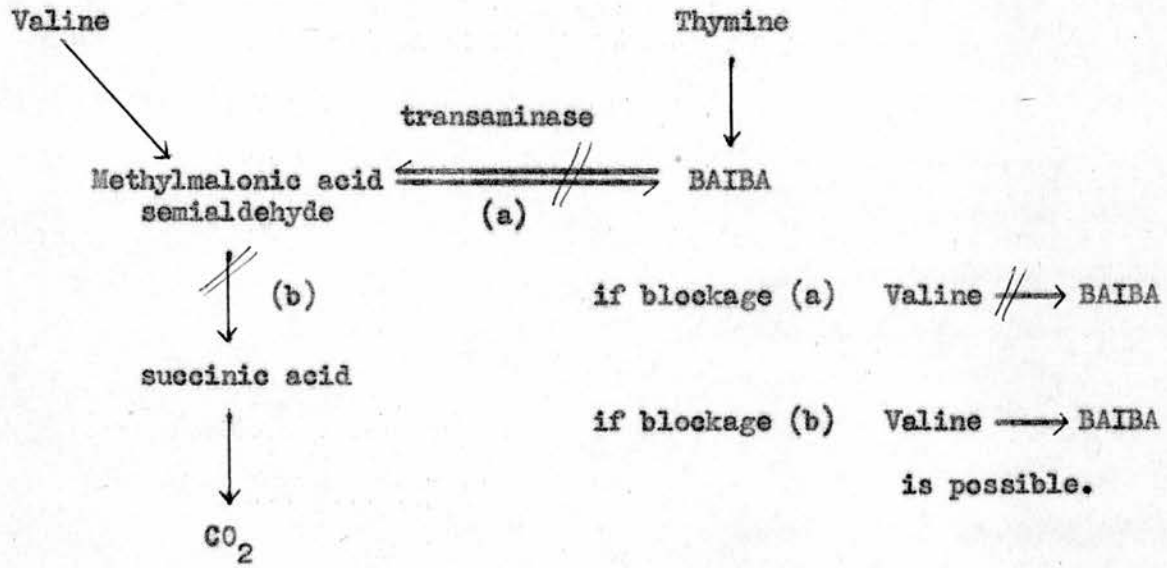
11.2 Other Metabolites.

(i) Valine Studies

It is intended to continue the valine studies, and if possible, administer valine to human subjects receiving radiotherapeutic treatment, and investigate what effect this has on BAIBA metabolism.

If the elevated excretion of BAIBA observed in the cases of "high excretors", subjects with active neoplastic disease and subjects exposed to ionising radiation, is due to the blockage of the transaminase enzyme system in BAIBA breakdown, then the catabolism of valine should have little influence on the excretion of BAIBA. However, if the block is

subsequent to methylmalonic acid semialdehyde, and assuming this pathway to be the principle means of valine breakdown, then valine should also contribute to the BAIBA excreted. Valine loading should facilitate this investigation.



This emphasises the importance of valine in the study of BAIBA metabolism.

(ii) Taurine Studies

Although urinary taurine levels are very much dependent on the dietary intake, this need not be a serious limitation in these studies involving hospitalised subjects. There is little doubt that hypertaurinuria is a sensitive and early indicator of ionising radiation damage and in view of the obvious importance of the sulphur atom in radiation chemistry

it is intended to continue the taurine studies under carefully controlled dietary conditions at a later date. The isolation of taurine by the standard Technicon method is sometimes difficult. A simple rapid method for taurine determination has been developed. This involves very short columns (30 × 0.63 cm) of cation exchange resin, and a system of water elution, resulting in complete integration of taurine and an analysis time of 30 min./ determination including regeneration. This method also appears to be suitable for cysteic acid and urea.

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