4. Catechol and adrenaline, which are sometimes used as reducing agents for the measurement of cytochrome oxidase activity, are not satisfactory for this purpose.

5. The concentration of phosphate buffer has a very important effect on the rate of oxidation of *p*-phenylenediamine and ascorbic acid by heart-muscle preparation.

6. It is probable that the added cytochrome *c* when attached to its enzyme cannot be readily reduced by ascorbic acid. It is suggested that the effect of the concentrations of cytochrome *c* and reducing agent on the rate of O_2 uptake can be explained by assuming that the rate of diffusion of cytochrome *c* to and from its enzyme may limit the rate of the overall reaction.

7. The effect of fluoride on the rate of O_2 uptake found by Borei is probably due not to combination of cytochrome *c* with fluoride, as suggested by Borei, but to an effect of fluoride on the particles of the enzyme preparation, causing an impairment of the catalytic activity of added cytochrome *c*.

8. The true cytochrome oxidase activity of Keilin & Hartree's heart-muscle preparation is expressed by a Q_{O_2} (based on a fat-free dry weight) of 3400 at 38°, which is much higher than previously reported.

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Studies on the Absorption of Proteins: the Amino-acid Pattern in the Portal Blood

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Although dietary protein is certainly the source of body protein, the steps by which it is transferred from the ingested food to the tissues are still incompletely known. In particular, there is considerable doubt about the form in which protein is absorbed from the gut into the portal blood stream, and the possible effect of the liver in altering the products of protein digestion on their way to the tissues. The tacitly accepted view is that the protein is completely broken down into free amino-acids in the gut. These are absorbed into the blood stream and carried to the tissues, each of which withdraws such amino-acids from the blood as it may require for the synthesis of its own proteins. This view is based

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mainly on the facts that the intestinal enzymes are able to break down proteins into amino-acids, and that after a protein meal the level of blood amino-acids rises (Van Slyke & Meyer, 1912). The rise in amino-acid concentration in the systemic blood after protein ingestion does not, however, prove that the amino-acids are the actual compounds absorbed from the gut, for, if the protein were distributed in the blood stream to the tissues in intact or only partially degraded forms, the tissues, in taking up these and converting them into the required new protein, would presumably have to discard amino-acids present in excessive proportions while retaining those required for the synthesis of new protein. The unwanted amino-acids would diffuse into the blood and cause a similar rise in the total amino-acid concentrations. That fragments of protein, larger than amino-acids, are absorbed is suggested by a considerable amount of indirect evidence (Verzar & McDougall, 1936). We cannot, therefore, draw conclusions from changes in the blood amino-acid levels unless we know which individual amino-acids are involved, and whether the portal blood levels are higher or lower than those in systemic venous blood.

Part of the difficulty in devising experiments to decide between the possible explanations is undoubtedly technical. Owing to practical difficulties in obtaining portal blood previous workers have usually limited their studies to the changes in systemic blood. Such results necessarily provide a precarious basis, at best, for inferences regarding the composition of the portal blood. Furthermore, it has been impossible in the past to carry out even the most approximate qualitative and quantitative analyses of most of the individual amino-acids in the blood, hence observations have been limited to determinations of total free amino nitrogen which give no indication of the composition of the amino-acid mixture. Even greater difficulties are met with in attempting to determine the blood levels of nitrogen carried in the form of peptides (Christensen, Decker, Lynch, Mackenzie & Powers, 1947); indeed, no specific method for their assay in blood is as yet available.

It was decided to apply the technique of paper-partition chromatography (Consden, Gordon & Martin, 1944) to the analysis of blood obtained during protein digestion. This technique permits the recognition of nearly all the amino-acids and peptides in a mixture, and, besides being of exceptional specificity, it also allows of a rough quantitative assessment of the amounts of each amino-acid present. By the use of the London cannula, portal blood can be drawn for comparison with systemic blood. By combining these techniques it was hoped to obtain more definite evidence than had been possible hitherto of the mechanism of protein absorption.

Ten technically satisfactory experiments are described in which dogs were given by mouth, casein, casein hydrolysate (Amigen), ground beef, human serum albumin, and dog whole plasma protein. The latter protein was given in order to test the possibility that homologous protein might be absorbed differently from foreign (i.e. heterologous) proteins.

Fortunately an exceptional opportunity arose to check the main trends of the results by submitting some of the samples to Dr H. N. Christensen for analysis for free, and in some cases also for combined, α-amino nitrogen. His results are quoted separately in the addendum to this paper.

METHODS

General

The dog's regular kennel diet was withheld from the morning of the day before until the experiment was over. In each experiment, when possible, 100 g. of protein were given. It was given as quickly as possible by stomach tube when fluid enough, by spoon, or by normal eating according to the nature of the meal. Portal and jugular blood samples were collected just before the protein administration and again 1, 2·5 and 5 hr. afterward. Each time 15 ml. were drawn and added to 0·2 ml. of 1% heparin solution. The blood was centrifuged and the plasma separated, usually within 15 min. of obtaining it. No sign of haemolysis was seen.

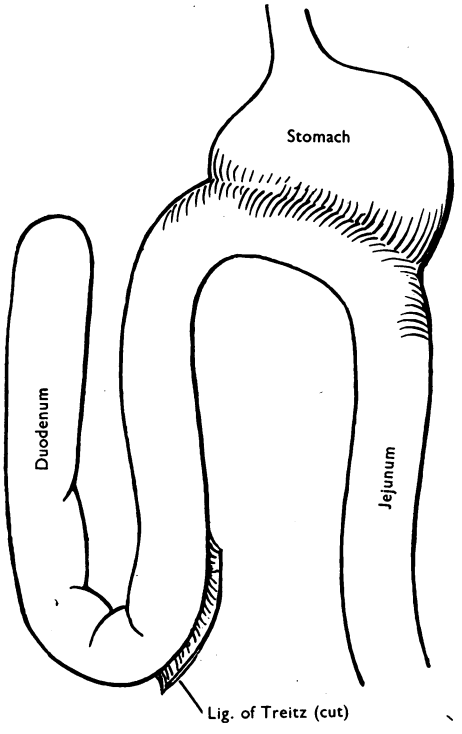

Fig. 1. Diagram showing the essentials of the partial gastrectomy operation

Operative procedures

Partial gastrectomy operation. Mongrel dogs were selected that averaged 15 kg. in weight. Under nembutal anaesthesia and through a left upper rectus muscle-splitting incision, the peritoneal cavity was entered. A classical Polya type of gastrectomy was performed with resection of about two thirds of the stomach, including the pylorus. The stomach was anastomosed to the first loop of the jejunum after mobilization of the duodenum with utilization of the full length of the gastric stoma, as indicated in the accompanying diagram (Fig. 1). The anastomosis was performed openly and silk technique was used throughout. The wounds were all closed in layers with interrupted four zero silk sutures.

Post-operatively the dogs were allowed water *ad lib.* the first day, milk and eggs until the fifth day, and then a soft cage diet of table scraps. The weight of the animals was followed carefully. Most animals lost 10–15% of their body weight and stabilized at this lower level. Blood analyses for total proteins, albumin-globulin ratio, haemoglobin, red cell count, and non-protein nitrogen were done at intervals. The animals appeared normal in every way. Their behaviour, appetite and activity were checked daily, and, in conjunction with the body weight, afforded a better index of the animals' condition than the blood analyses.

Insertion and operation of the London cannula. London's (1935) original technique was used in the first 2 experiments. This was a two-stage operation where the first stage consisted of painting the portal vein with iodine solution, and immobilizing it by sewing to the side of the adjacent part of the inferior vena cava. Two weeks later the second stage was performed, namely suturing the cannula to the painted area on the portal vein, and then wrapping it around with an elongated mass of excised free omentum. The cannula was made from a no. 16 lumbar puncture needle. A small perforated cross-piece was welded on the distal end as indicated in the accompanying drawing (Fig. 2). Varying lengths of cannulae could be used, depending on the size of the animal and the location of the portal vein. The cannula was brought out between the lower ribs through a stab wound, care being taken not to open the pleural reflexion. In the third and all subsequent experiments, a one-stage procedure was used omitting the iodine painting and the immobilization of the portal vein, but otherwise operating as above. Only one complete failure to draw portal blood occurred in the use of this simplified technique. In addition, a correctly placed cannula, that had operated satisfactorily on one occasion, failed to draw portal blood when used for another experiment (see Exp. 11). The position of the cannula was always checked carefully by post-mortem examination of the animals. In the X-ray photograph (Fig. 3), the general position of the cannula can be seen. The thorotrast injected into the portal vein and faintly outlining the portal radicals in the liver also serves to illustrate the method of withdrawing blood from the portal vein.

The cannula was anchored at the skin with an encircling suture and a metal cup was placed over the external stab wound. A jacket was then tied snugly around the trunk of the animal to prevent the cup from slipping, and to prevent the animal from tearing the bandages and cannula with his teeth. Liberal use of adhesive tape was useful for securely fastening the cup and jacket.

Post-operatively, the animals showed little reaction to the procedure. They were able to move with freedom in their cages. A full diet was usually tolerated the next day though, if not, milk and eggs were supplied. The dressings were not changed until the time of the first experiment, 5 or 6 days afterwards.

It is intended to publish the technique of cannula insertion in more detail elsewhere.

Chromatographic analysis of blood

Preparation of blood samples for paper chromatography. Earlier work on the application of paper chromatography to protein-containing solutions had shown that although traces of protein (up to 0·5%) had little effect on the subsequent analysis, larger quantities interfered seriously, this being apparently related to the viscosity of the protein solution which acted as a mechanical barrier to the smooth soakage of the solvents along the filter paper. It was decided that ultrafiltration ought to be the best method for the removal of blood protein, and a few trials showed that this could be readily adapted to deal with the blood samples in these experiments. The following standard procedure was always used. The apparatus was that described by Greenberg & Gunther (1929). The following modification of the method of preparing the collodion sac was used: collodion (U.S.P.) to which 4% (v/v) of ethylene glycol had been added, was poured into a 15 ml. centrifuge tube which was then emptied for 30 sec. while holding it at an angle of 30° to the horizontal with continual rotation. It was then clamped upside down in a vertical position for 10 min. It was again filled quickly with the collodion and emptied as before. It was now, however, drained vertically overnight. It was then held with the open end upwards, and two drops of the collodion allowed to drop down the middle to reach the tip directly. This was then dried off quickly by a light blast of air and the tube held upside down for a few hours. This extra reinforcement of the thickness of the tip was a very necessary precaution against subsequent tearing. The collodion bag now lining the centrifuge tube was ready for use. If not used at once it could be kept safely in this situation for several months without apparently affecting its final efficiency. When required, it was easy to pull it out of the tube and attach it to the apparatus. Only a very slight contamination of the product with the ethylene glycol would occur as most of the latter drained off during the drying and contraction of the collodion film. No more than 1 in 20 of these sacs would burst or develop leaks during the ultrafiltration. They usually burst if the pressure was raised to 250–300 mm. of Hg. These membranes allowed inulin (mol. wt. *c.* 5000) to filter through quantitatively. Cytochrome *c* (mol. wt. 13,000) was, however, completely retained.

Ultrafiltration procedure. The ultrafiltration of the heparinized plasma was carried out at a pressure of 150–200 mm. of Hg, the whole apparatus being immersed in an ice bath. About half of the volume of plasma taken would be collected as crystal-clear protein-free filtrate in 8 hr. Very little more came through in a longer time, although, for reasons of convenience, the apparatus was usually run overnight. The ultrafiltrates were kept at 4° for the few days while they were awaiting chromatographic analysis. This scale of operation was chosen for convenience of manipulation, to eliminate to a negligible degree the possible loss of substances by absorption on to the collodion, and because other analytical methods requiring larger volumes were envisaged. As far as the paper chromatography was

concerned the whole analysis could have been carried out on less than one tenth of the volumes taken above.

Paper chromatography method. Volume of ultrafiltrate taken. The standard volume of 125 μl. was taken throughout. As this was too large a volume to apply directly to the paper it was always evaporated almost to dryness on a watch glass by blowing air over it at room temp. by means of an electric fan. The residue was then transferred to the paper followed by 3 washings of the watch glass. This volume was chosen as, by its use, a normal blood with NH_2-N conc. of 4 mg./100 ml. would produce on the chromatogram up to 12 weak spots from the amino-acids present in greatest amount. An increase of 50% in NH_2-N conc. would be easily picked up by the appearance of further amino-acids, as well as by a strengthening of those seen before. The ninhydrin colour reaction as carried out on the paper becomes less sensitive at much higher concentrations of amino-acids and an increase of 50% may, therefore, not be observed in such a case. To compare high concentrations less ultrafiltrate would have to be taken to bring the spots down to the more sensitive range.

Procedure. Two-dimensional chromatograms were used almost exclusively. Phenol was used as the first solvent, in the presence of a trace of NH_3 vapour, and collidine-lutidine mixture in the presence of a trace of diethylamine as the second. Further details are given by Dent (1948).

Distortions on the chromatogram. The final positions occupied by aspartic and glutamic acids on the chromatograms were always abnormal and the spots in question partly distorted into streaks. This phenomenon was due to the presence of three substances which appeared as large yellow spots and which took precedence in the occupation of certain areas of the paper with the result that the amino-acids were left to surround the margins of these areas (Figs. 4–9). Glutamine was sometimes affected in a similar manner. The interference was always similar in character. Once, therefore, the new positions of the amino-acids had been determined, it was still possible to carry out satisfactory identifications although the rough quantitative estimations were somewhat jeopardized.

Recent work by Westall (1948) suggests that two of the yellow spots (*A* and *C* in Fig. 7) are probably due to inorganic acid and to sodium phenoxide, respectively, formed by the separation on the chromatograms of the ions of inorganic salts. The present writers have recently applied the de-salting device of Consden, Gordon & Martin (1947) to plasma ultrafiltrates and have obtained undistorted chromatograms from as much as 625 μl. This represents a considerable advance on the technique as used in the present work.

Identification of amino-acids. This was carried out by the usual methods (Dent, 1948), i.e. careful position matching with markers followed by confirmation that the substances were stable to hydrolysis, and, except for methionine and cystine which are oxidized, were also stable to treatment with H_2O_2. Once one sample of fasting dog blood had been checked in this way the identification thus obtained was assumed to apply to the spots given by fasting blood from other dogs. In the blood drawn after the proteins had been fed, only the serial sample which contained the highest NH_2-N concentration was checked, and this only by prior hydrolysis and H_2O_2 treatment. It was not considered necessary to re-check all the amino-acids with markers in such cases. Leucine and isoleucine, which overlap on the chromatograms, were always considered together under the name 'leucines'. It should be emphasized that the substance, moving to a position close to and just below alanine in the chromatograms as represented in this paper, was always shown to be glutamine and not the peptide 'under-alanine' (Dent, 1947), which also moves to the same place.

Method of recording strengths of spots. The colour strength of each spot was recorded against an arbitrary colour chart divided into 10 parts. A strength of 1 represented a very weak purple colour, 10 an intense purple. When the colour could not be seen directly, but was just visible when the paper was viewed by transmitted light, it was called <1. Colour strength of greater than 10 (recorded as >10) or much greater than 10 ($\gg 10$) were well into the range where the colour reaction was much less quantitative. The strengths of the yellow ninhydrin colours such as are given by proline were assessed by plus signs.

Detection of peptides. For the purpose of this paper a substance is called a 'peptide' if it moves to a characteristic position on the chromatogram not corresponding to a known substance, if it gives a typical ninhydrin colour reaction, and if it is destroyed on acid hydrolysis (see below) with simultaneous liberation of free amino-acids. Isolation and identification of peptides was considered to be beyond the scope of this paper.

Whether or not peptides are detected when they are present in only small amount depends entirely on whether they are present as only a few distinct members, say 3 or 4 dipeptides, or as a very large number of di-, tri-, and polypeptides. In the latter case a larger amount of peptide N could be undetected if it were widely distributed about the paper, each peptide being present in too small a quantity to give a visible ninhydrin colour. The only way to detect this would be by observing an otherwise unaccountable increase in free amino-acids after acid hydrolysis. Such an increase could also occur, however, from other sources, e.g. from acyl derivatives of amino-acids such as hippuric acid. There is some indirect evidence at least in the cases of aspartic and glutamic acids (Dent, 1947) for the existence of further members of this series. The finding of such an increase in amino-acids on hydrolysis is more safely described, therefore, as being due to 'bound amino-acids'. A hydrolysis was performed on at least one of the ultrafiltrates of the blood samples from each experiment whether or not more direct evidence of the presence of peptides had been obtained. It was estimated that bound amino-acids, if involving all the amino-acids in similar relative proportions to their occurrence in the free state, could be present containing up to 20% of the total NH_2-N without being detectable on the chromatograms. On the other hand, if the bound amino-acids concerned only one or two members then the method would be far more sensitive. These opinions are based on the results of experiments with synthetic peptides and with proteins partly hydrolyzed by enzymes, acids and alkalis.

Acid hydrolysis of samples. The sample (125 μl.) was mixed with an equal volume of concentrated HCl, sealed in a capillary tube and immersed in boiling water for 24 hr. It was then transferred to a watch glass, evaporated to dryness at 60°, and the residue washed on to the filter paper, with about 30 μl. of water, applied in several lots.

Brief summary of each dog experiment

Exp. 1 (dog 46-1). Casein, 28 January 1947; wt. 16 kg.; first stage of cannula insertion, 10 January 1947; second stage 24 January 1947. Casein (100 g.) damped with about

200 ml. of water was fed by spoon. Blood samples were apparently collected satisfactorily. The dog was killed after the 5 hr. period. The stomach was found to be full of casein, and also contained some bandages, presumably from the dressings. It was thought that the latter might have produced some obstruction to the further passage of the casein. In view of the lack of absorption of the casein the blood samples were rejected.

Examination of the cannula insertion showed that the inferior vena cava contained a series of recent punctures while the adjacent portal vein was intact. Clearly the 'portal' blood samples taken in this experiment were really of systemic origin.

Exp. 2 (dog 46-10). Casein, 17 February 1947; wt. 11 kg.; first stage of operation 28 January 1947; second stage 12 February 1947. Casein (100 g.) fed by spoon. The blood samples were drawn without difficulty. The dog was killed after the 5 hr. period. The stomach was found to contain 37 g. of apparently unchanged casein. It is possible, therefore, that the bandages were not the cause of the delay in stomach emptying found in Exp. 1. It was as a result of this that the decision to do the prior partial gastrectomy operation was taken. The cannula had been working very well in this experiment and only the portal vein had been pierced by the sampling needle.

Exp. 3 (dog 46-32). Dog plasma, 24 March 1947; wt. 9 kg.; partial gastrectomy operation 3 June 1946; cannula inserted 18 March 1947. (From now on this was done by the one-stage method, see p. 320.) Freeze-dried (lyophilized) dog plasma protein (100 g.) made up to 400 ml. with water was given by stomach tube. Considerable gastro-intestinal disturbance resulted. The dog vomited a large amount 10 min. afterwards. This was given back to the dog. A further vomit 40 min. later was also given back by tube. A small amount of diarrhoea occurred 1·5 hr. later. This was rejected. It was estimated that not more than 25 g. in all of the original protein could have been lost during these incidents. The blood samples were taken very satisfactorily. It was, therefore, decided to use the same dog for the next experiment, and to repeat later the protein feeding with dialyzed plasma, as the gastro-intestinal upset might have been due to the high salt content of the concentrated plasma.

Exp. 4 (dog 46-32). Casein 28 March 1947; same dog as exp. 3; wt. now 9·6 kg. Casein (100 g.) fed by spoon. Blood samples taken satisfactorily, although on one occasion when perhaps the needle was inserted a little farther than usual, pure bile was withdrawn in the syringe. Urine passed at 1·5 hr. was collected. At 4·5 hr. the dog vomited 250 ml. of bile-stained liquid, containing much mucus and a small amount of casein. The dog was killed after the 5 hr. period. The cannula was found to have been working satisfactorily and only portal blood had been drawn through it. The gall bladder and bile duct were distended, presumably as the result of a stricture produced by adhesions around the cannula. There was therefore some degree of biliary obstruction, and it was easy to see how the sample of bile had been accidentally withdrawn. The stomach and intestines were empty and normal in appearance. The liver also appeared normal; sections were taken and found to be normal microscopically. Blood analyses showed: icterus index, 9 units; bilirubin, 0·13 mg./100 ml. (direct) and 0·42 mg./100 ml. (total).

Exp. 5 (dog 46-178). Casein, 16 June 1947; wt. 15·5 kg.; partial gastrectomy operation 13 January 1947; cannula inserted 11 June 1947. Casein (100 g.) fed by spoon. Blood samples taken satisfactorily. Dog was well throughout the experiment. After the 5 hr. period had been passed the dog was X-rayed while thorotrast was being injected through the cannula. The picture (Fig. 3) confirmed that the needle was inserted into the portal vein. The dog was then killed. The cannula was seen to be well placed and the inferior vena cava unpunctured. The stomach and intestines were empty except for a few small particles of undigested casein in the upper part of the small intestine.

Exp. 6 (dog 47-11). Dog plasma, 28 June 1947; wt. 13·5 kg.; partial gastrectomy 20 January 1947; cannula inserted 23 June 1947. Dialyzed and freeze-dried dog plasma protein (100 g.) was made up to 400 ml. with water and given by stomach tube. Blood samples taken satisfactorily. Portal and jugular blood was also taken at 8 hr. in this experiment. This animal did not vomit, but 1·5–2·25 hr. after it had been fed by tube it had some considerable diarrhoea. The dog excreted material which closely resembled the protein solution originally fed. Fortunately, however, the dog avidly ate up at once everything he had passed, so that only a few g. protein were lost. The dog was kept for a further experiment.

Exp. 7 (dog 47-11). Amigen, 3 July 1947; same dog as in Exp. 6; well since then. Hydrolyzed casein (Amigen, 100 g.) stirred with 300 g. of warm water was given by stomach tube. The dog appeared well throughout the experiment. The blood samples were taken satisfactorily and, in addition, a sample of urine passed at 4 hr. The dog was killed after the 5 hr. samples were taken. At post mortem the correct position of the cannula was confirmed. The gut was apparently empty.

Exp. 8 (dog 47-60). Dog plasma, 23 July 1947; wt. 10 kg.; partial gastrectomy 18 February 1947; cannula inserted 17 July 1947. The dog was given, by stomach tube in two equal portions 15 min. apart, 800 ml. of heparinized dog plasma. This had been drawn from donor dogs two days previously and kept frozen solid. It contained at least 50 g. of dry plasma protein. From the 0·5 hr. period after feeding to 2 hr. after, this dog vomited intermittently. The vomit was collected each time and given back by tube, at the most about 50 ml. of the original 800 ml. being lost. There was no diarrhoea. The blood samples were taken satisfactorily. After the last sample the dog was killed. At post mortem the cannula was found to be correctly placed. The stomach and small intestine were empty, but the large intestine contained some semi-liquid faecal material. The entire gut from stomach to rectum was carefully squeezed out. The material measured 65 ml., appeared to be only faeces and contained 1·6 g. of total N, corresponding to about 10·0 g. of total protein (N × 6·25).

Exp. 9 (dog 47-48). Human-serum albumin, 1 August 1947; wt. 11 kg.; partial gastrectomy 24 February 1947; cannula inserted 26 July 1947. The dog was given by stomach tube 50 g. human albumin (200 ml. of 25% solution) at 0 hr. and another 50 g. 0·5 hr. later. The albumin was described as 'concentrated salt-poor human-serum albumin' (Squibb and Sons, New York). It contained 1% of DL-acetyltryptophan as stabilizer. The dog tolerated this dosage very well, appearing lively and well throughout, in marked contrast to the gastro-intestinal upset in the experiments in which dog plasma was fed. Blood samples were taken satisfactorily. Urine was passed at the start of the experiment, and a sample collected when the bladder was emptied at 5 hr. The dog was kept for further experiments.

Exp. 10 (dog 47-48). Denatured dog plasma, 6 August 1947; same dog as in Exp. 9. Heat-denatured plasma protein (80 g.) obtained from donor dogs was fed by spoon quite satisfactorily. About 0·5 hr. later the dog vomited what appeared to be the entire amount of protein. Half of this was fed back to the dog, but was again vomited shortly after. In view of the apparently complete intolerance to this substance the experiment was discontinued.

Exp. 11 (dog 47-48). Ground beef (Hamburger), 7 August 1947; same dog as in Exp. 10; wt. 10·5 kg. Raw ground beef (500 g., *c.* 105 g. of protein) with the fat stripped off as much as possible before mincing, was fed. 0·5 hr. later the dog vomited some apparently unchanged meat mixed with mucus. Some of this was eaten again quickly. The remainder weighed 192 g. and was rejected. Therefore at least 308 g. containing about 65 g. of protein were retained. The blood samples were taken satisfactorily. After the 5 hr. period the dog was killed. The post mortem showed that the end of the cannula had slipped from the portal vein. The portal vein showed some brown pigmented spots where it had presumably been punctured the week before (Exp. 9). On the other hand, the inferior vena cava showed recent red staining undoubtedly over the sites of recent needle pricks. The gut was found to be fairly full of dark smelly liquid. This was considered to be a normal residue after eating meat.

Exp. 12 (dog 47-67). Ground beef, 14 August 1947; wt. 18 kg.; partial gastrectomy 8 April 1947; cannula inserted 8 August 1947. Raw ground beef (500 g.) as fat free as possible was fed. The blood samples were taken satisfactorily. The dog was lively and well throughout the experiment. It was saved for further trials.

Exp. 13 (dog 47-67). Human-serum albumin, 16 August 1947; same dog as in Exp. 12. Dog was fed 400 ml. of 25% human albumin (same material as in Exp. 9) by stomach tube. This was well tolerated, the dog appearing lively throughout. Blood samples taken satisfactorily. Urine was collected when the bladder was emptied at the beginning of the experiment, and again after 4 hr.

The dog was saved for a further experiment on 20 August 1947 (not reported here) in which glutamic acid was fed. After this experiment the dog was killed. A post mortem then gave unequivocal evidence that the cannula had been correctly placed.

RESULTS

Amino-acid analyses by one-dimensional chromatograms. This method was used as a rough sorting test to detect potentially interesting fluids. When one was found, e.g. the urine after feeding human albumin, the fluid was tested further on two-dimensional chromatograms (Tables 2 and 3) so there is no need here to state the result of the rough test. In the urines obtained after feeding casein (Exps. 2 and 4) there were no abnormal amino-acids detectable in the one-dimensional chromatograms so no further tests were done. Unfortunately, no urine was tested after the feeding of dog plasma, although it could hardly be expected to have been abnormal, owing to the normal blood levels of amino-acids.

Amino-acid analyses using two-dimensional chromatograms. These are summarized in Tables 1–3. A series of original chromatograms is much easier to interpret than the tables. A few chromatograms are, therefore, reproduced photographically or as diagrams in Figs. 4–15. Tryptophan and hydroxyproline, although easily detectable if present in sufficient quantity, have not been seen in any of the blood samples. They are not, therefore, mentioned in the tables. Asparagine is also omitted although it has been found once (see Fig. 11). There is considerable doubt as to the identity of citrulline. The spot given in this position by normal blood has the characteristic reddish purple colour of citrulline, although there is not enough present to confirm the identity by the spot test with *p*-dimethylaminobenzaldehyde (Dent, 1948). It is possible, therefore, that the spot could have been due to β-alanine, which moves to a similar position, although it usually gives a ninhydrin colour having a bluer shade. In one hydrolyzed urine (Exp. 13, 0–4 hr.) the spot was certainly due chiefly to β-alanine as there was sufficient quantity present to do the chemical test. Reasons mentioned in the Discussion suggest that the increase in strength of the spot so often found after acid hydrolysis might always be due to β-alanine.

The order of the amino-acids in Tables 1–3 is roughly that in which they are spread out on the paper. This order soon becomes familiar to workers using the chromatograms. The numbers under each substance refer to the colour strength of the ninhydrin reactions on the arbitrary scale of 10. They are of relative value only. The strength of the aspartic acid spot was often difficult to judge owing to the distortion always present.

Analyses for α-amino nitrogen by chemical methods. The following results were obtained by Dr H. N. Christensen with the methods described in the addendum to this paper.

Dog (*Exp.* 4). Analysis of ultrafiltrate of vomit ejected 4·5 hr. after giving the 100 g. of casein: total α-amino nitrogen, 440 mg./100 ml.; free α-amino nitrogen, 69 mg./100 ml.

Dog (*Exp.* 13). Analysis of urine passed just before human albumin was given, in mg. α-amino nitrogen/100 ml.: total 63·1, free 16·0, bound 47·1. The next urine specimen was passed 4 hr. after the experiment had begun and contained: total 417, free 342, bound 75.

Electrophoretic analysis of portal plasma after feeding human-serum albumin. Human-serum albumin injected intravenously into dogs can be readily detected in the plasma by electrophoretic means many hours after the injection (McKee & Alling, 1946). Dr Alling kindly examined in this way the portal plasma 2·5 hr. after feeding human-serum albumin in Exp. 13. No peak corresponding to

the human albumin was seen and the portal plasma at 2·5 hr. matched in every respect the fasting sample at 0 hr. By this method, it was estimated that at least 10 g. of circulating human-serum albumin could be detected. Hence of the 100 g. fed little if any could have been absorbed intact.

Specific gravity of portal plasma. As a rough check on plasma protein content the specific gravity of the specimens of portal and jugular plasma was determined by the copper sulphate method (Phillips, Van Slyke, Dole, Emerson, Hamilton & Archibald, 1945). No significant change in specific gravity was found on any of the specimens taken during Exps. 5 (casein), 6 (dog plasma), and 9 (human-serum albumin).

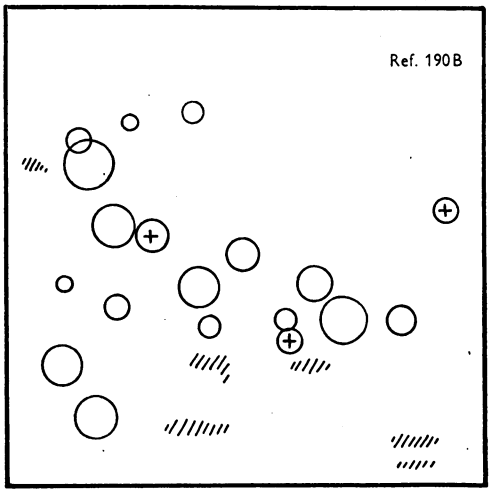

Fig. 10.

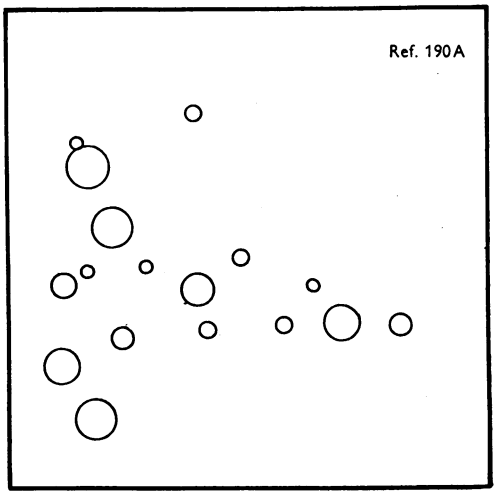

Fig. 12.

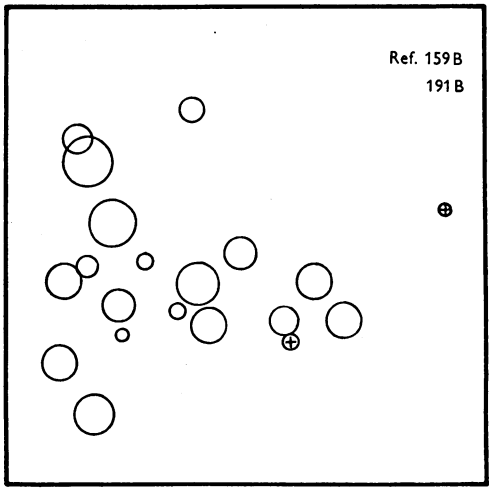

Fig. 11.

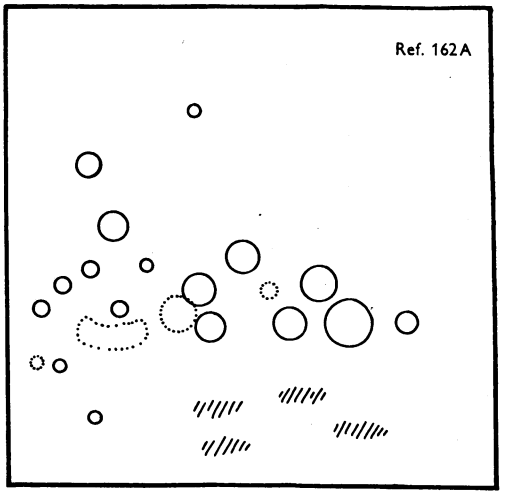

Fig. 13.

Figs. 10–13. Diagrams of chromatograms obtained during the feeding of casein hydrolysate (Amigen). In these figures, the size of the spots has been drawn to represent the strength of the ninhydrin colour. The identifications can be made from Fig. 7. Aspartic and glutamic acids are, however, shown in these diagrams in their true positions, not in the distorted positions as in Fig. 7. The three further spots in Fig. 10 (see also Fig. 11) marked in the centre with crosses are, from right to left, cysteic acid (from cystine), asparagine, and methionine sulphone (from methionine). The spots outlined with a dotted line or shown by shading represent 'peptides'. The materials run on each chromatogram were: Fig. 10, 300 μg. of Amigen; Fig. 11, 125 μl. of the 1 hr. portal blood ultrafiltrate; Fig. 12, 125 μl. of the 1 hr. jugular blood filtrate; Fig. 13, 25 μl. of the urine secreted in the first 4 hr. of the experiment.

Table 1. *Levels of amino-acids in body fluids*

(Figures indicate arbitrary units, see p. 321.)

Sample analyzed	Exp. 2. 100 g. casein (dry wt.) fed								Exp. 3. 100 g. dog plasma protein (dry wt.) fed. About 25 g. subsequently vomited					Exp. 4. 100 g. casein (dry wt.) fed. About 37 g. vomited at 4·5 hr.						
	Plasma filtrate	Plasma filtrate	Plasma filtrate	Plasma filtrate	Plasma filtrate	Plasma filtrate (hydrolyzed)	Plasma filtrate	Plasma filtrate (hydrolyzed)	Plasma filtrate	Plasma filtrate	Plasma filtrate	Plasma filtrate (hydrolyzed)	Plasma filtrate	Plasma filtrate	Plasma filtrate	Plasma filtrate	Plasma filtrate (hydrolyzed)	Plasma filtrate	Vomit filtrate *	Vomit filtrate (hydrolyzed) †
Time of sample (hr.)	0	1	1	2·5	5	5	5	5	0	1	2·5	2·5	5	0	1	2·5	2·5	5	4·5	4·5
Portal or jugular	P	P	J	P	P	P	J	J	P	P	P	P	P	P	P	P	P	P	.	.
Cysteic acid (from cystine)	.	.	.		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Aspartic acid		1	.	1	?	?	?	?	.	.	.	.		.	.	.	.	.	1	7
Glutamic acid	3	1	3	1	5	7	2	6	3	3	3	4	3	3	4	5	8	4	8	>10
Serine	1	2	1	2	3	3	.	1	.	.	.	<1	.	<1	3	4	1	<1	2	5
Taurine	.	<1	.	.	.	<1	.	.	.	.	.	.	.	.	.	.	.	.	<1	<1
Glycine	4	2	2	2	4	4	2	3	.	3	2	1	2	3	4	1	2	<1	<1	6
Threonine	3	3	2	3	4	3	.	3	.	.	2	<1	.	.	2	4	3	1	1	5
Alanine	4	4	5	3	7	7	5	6	4	4	3	4	3	4	8	6	6	5	5	10
Glutamine	4	4	5	4	6	.	5	.	2	3	2	.	3	2	4	4	.	3	5	.
Tyrosine	<1	1	<1	2	2	2	<1	<1	.	.	<1	.	.	<1	2	3	3	<1	2	6
Citrulline and/or β-alanine	.	.	.	.	.	1	.	1	.	.	.	<1	.	.	.	.	1	.	1	<1
Histidine		.	.	.	.	1	.	<1	.	.	.	.	.	.	<1	1	.	.	.	4
α-Amino-*n*-butyric acid	1	1	1	1	1	1	1	1	<1	<1	<1	<1	<1	<1	1	2	2	1	<1	<1
Methionine sulphone (from methionine)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.
Valine	4	6	5	7	8	8	6	6	4	4	4	4	5	3	7	8	8	7	4	9
Methionine sulphoxide	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	2	5
γ-Aminobutyric acid	<1	.	1	.	1	3	<1	2	.	.	.	<1	.	<1	1	3	2	5	?	?
Lysine	<1	2	<1	3	4	3	1	2	<1	<1	<1	<1	<1	<1	2	3	2	1	<1	6
Phenylalanine	.	2	.	3	.	.	.	.	1	2	.	<1	.	.	3	3	1	.	.	7
Leucines	4	6	5	8	8	8	6	6	4	4	4	3	5	4	8	9	9	6	8	>10
Fast aminobutyric acid	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Proline	.	+	<+	++	+++	+++	+	+	.	.	.	.	.	.	++	+++	++	+	.	+++
Arginine	1	<1	<1	1	3	2	<1	1	1	1	<1	.	<1	1	1	2	2	<1	1	5

* Large numbers of peptides also seen.

† All the peptides have now disappeared. Methionine is seen partly overlapping leucine. There is also an unidentified substance 'fast arginine' (see Dent, 1947).

Table 2. *Levels of amino-acids in body fluids*

(Figures indicate arbitrary units, see p. 321.)

	Exp. 5. 100 g. casein (dry wt.) fed					Exp. 6. 100 g. dog plasma protein (dry wt.) fed					
Sample analyzed	Plasma filtrate	Plasma filtrate	Plasma filtrate (hydrolyzed)	Plasma filtrate	Plasma filtrate	Plasma filtrate	Plasma filtrate	Plasma filtrate	Plasma filtrate (hydrolyzed)	Plasma filtrate	Plasma filtrate
Time of sample (hr.)	0	1	1	2·5	5	0	1	2·5	2·5	5	8
Portal or jugular	P	P	P	P	P	P	P	P	P	P	P
Cysteic acid (from cystine)	.	<1	.	.	.	.	.	.	.	.	.
Aspartic acid	.	.	.	.	.	.	.	.	.	.	.
Glutamic acid	2	6	8	6	2	1	1	<1	6	<1	<1
Serine	1	6	3	6	3	1	1	.	<1	<1	<1
Taurine	3	3	1	2	<1	<1	<1	.	.	.	.
Glycine	1	2	3	2	<1	<1	<1	.	<1	<1	<1
Threonine	.	4	4	4	1	.	.	.	.	.	.
Alanine	6	8	9	8	6	3	3	1	4	1	1
Glutamine	4	6	.	7	4	3	3	2	.	2	2
Tyrosine	.	5	4	5	2	.	.	.	.	.	.
Citrulline and/or β-alanine	1	1	2	1	.	.	.	.	1	.	.
Histidine	.	.	4	.	.	.	.	.	.	.	.
α-Amino-*n*-butyric acid	<1	2	<1	2	2	<1	<1	<1	1	<1	<1
Methionine sulphone (from methionine)	.	1	.	.	.	.	.	.	.	.	.
Valine	6	9	8	8	7	5	5	5	5	4	2
Methionine sulphoxide	.	3	4	3	2	?	?	.	.	.	.
γ-Aminobutyric acid	.	<1	.	.	.	.	.	.	.	.	.
Lysine	1	7	7	6	4	.	<1	.	1	.	.
Phenylalanine	.	3	3	2	.	.	.	.	.	.	.
Leucines	1	9	9	8	7	3	2	2	3	1	1
'Fast aminobutyric acid'	<1	1	.	<1	<1	<1	<1	<1	1	<1	<1
Proline	<+	+++	++	++	+	.	.	.	.	.	.
Arginine	2	6	7	5	3	<1	<1	.	2	.	1

	Exp. 7. 100 g. casein hydrolysate (Amigen) (dry wt.) fed								Exp. 8. 50 g. dog plasma protein (dry wt.) fed				
Sample analyzed	Plasma filtrate	Plasma filtrate *	Plasma filtrate (hydrolyzed) †	Plasma filtrate	Plasma filtrate	Plasma filtrate	Urine ‡	Urine (hydrolyzed) §	Plasma filtrate	Plasma filtrate	Plasma filtrate	Plasma filtrate (hydrolyzed)	Plasma filtrate
Time of sample (hr.)	0	1	1	1	2·5	5	0–4	0–4	0	1·5	3	3	5·5
Portal or jugular	P	P	P	J	P	P	.	.	P	P	P	P	P
Cysteic acid (from cystine)	.	<1	.	.	.	.	.	.	.	.	.	.	.
Aspartic acid	.	.	?	2	.	.	2	4	.	.	.	.	.
Glutamic acid	1	6	10	6	5	3	9	⩾10	<1	<1	2	6	2
Serine	<1	6	6	<1	5	1	6	6	<1	<1	1	<1	<1
Taurine	1	<1	<1	.	.	.	.	.	<1	.	<1	.	<1
Glycine	1	4	5	1	4	<1	5	7	<1	<1	<1	<1	<1
Threonine	.	5	6	1	6	4	5	6	.	<1	1	1	<1
Alanine	6	8	10	5	7	4	5	8	2	3	4	3	4
Glutamine	4	6	.	1	3	2	4	.	2	2	2	.	2
Tyrosine	.	3	3	1	3	1	<1	1	.	.	<1	<1	<1
Citrulline and/or β-alanine	<1	1	4	.	1	1	6	4	.	.	.	<1	.
Histidine	.	.	3	.	<1	.	.	1	.	.	.	.	.
α-Amino-*n*-butyric acid	1	1	1	<1	1	1	.	<1	.	.	<1	<1	<1
Methionine sulphone (from methionine)	.	.	.	.	.	.	.	.	.	.	.	.	.
Valine	4	9	10	7	10	8	4	4	3	3	6	5	6
Methionine sulphoxide	.	5	4	2	5	4	1	2	.	.	<1	.	.
γ-Aminobutyric acid	1	<1	.	.	.	.	.	1	<1	<1	<1	<1	<1
Lysine	<1	7	9	7	8	5	1	<1	<1	<1	2	2	2
Phenylalanine	.	4	4	<1	3	2	.	<1	.	.	<1	<1	<1
Leucines	3	10	10	8	10	7	3	4	2	2	5	5	5
'Fast aminobutyric acid'	1	2	.	<1	<1	<1	.	.	.	<1	<1	.	<1
Proline	<+	+++	++	++	++	+	+	++	.	.	<+	.	<+
Arginine	1	6	8	6	6	3	<1	<1	1	1	2	2	2

* Trace asparagine seen.
† Two new spots also seen.
‡ Many peptides also seen.
§ One new spot also seen.

Table 3. *Levels of amino-acids in body fluids*

(Figures indicate arbitrary units, see p. 321.)

	Exp. 9. 100 g. human serum albumin (dry wt.) fed							Exps. 11 and 12. 105 g. ground beef fed (dry wt.)						Exp. 13. 100 g. human serum albumin (dry wt.) fed							
								Exp. 11 (35 g. of protein vomited)	Exp. 12												
Sample analyzed	Plasma filtrate	Plasma filtrate	Plasma filtrate	Plasma filtrate (hydrolyzed)	Plasma filtrate	Urine *	Urine (hydrolyzed)	Plasma filtrate	Plasma filtrate	Plasma filtrate	Plasma filtrate	Plasma filtrate (hydrolyzed)	Plasma filtrate	Plasma filtrate	Plasma filtrate	Plasma filtrate	Plasma filtrate	Plasma filtrate (hydrolyzed)	Plasma filtrate	Urine ‡	Urine §	Urine (hydrolyzed) ‖
Time of sample (hr.)	0	1	2·5	2·5	5	0–5	0–5	5	0	1	2·5	2·5	5	0	1	2·5	2·5	5	0	0–4	0–4
Portal or jugular	P	P	P	P	P	.	.	I.V.C.†	P	P	P	P	P	P	P	P	P	P	.	.	.
Cysteic acid (from cystine)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Aspartic acid	.	.	.	1	.	2	3	.	.	.	.	2	.	.	1	4	6	.	.	6	2
Glutamic acid	1	1	4	9	5	>10	>10	5	3	3	4	6	4	1	4	8	9	6	<1	⩾10	⩾10
Serine	2	<1	2	2	.	<1	4	<1	2	.	<1	3	.	.	<1	2	3	.	<1	6	4
Taurine	.	.	.	.	.	.	<1	.	<1	.	.	<1	.	.	.	.	<1	.	.	.	1
Glycine	.	.	<1	1	.	<1	8	.	.	.	<1	4	<1	.	1	2	2	.	.	5	8
Threonine	<1	<1	3	3	<1	<1	6	<1	<1	.	2	1	1	.	1	5	5	2	.	7	7
Alanine	3	1	5	7	3	4	8	5	4	2	7	7	5	3	5	8	9	5	1	9	10
Glutamine	3	2	2	.	1	.	.	.	.	.	<1	.	.	.	1	<1	.	1	.	.	.
Tyrosine	.	.	3	5	1	.	<1	<1	.	.	<1	2	<1	.	1	4	5	1	.	4	4
Citrulline and/or β-alanine	.	.	.	1	.	.	4	.	.	.	.	1	.	.	.	.	3	.	.	?	8
Histidine	.	.	.	1	.	.	.	.	.	.	.	3	.	.	.	.	1	.	.	.	8
α-Amino-*n*-butyric acid	.	<1	1	2	1	.	.	2	<1	<1	.	1	.	.	.	<1	<1	<1	.	.	1
Methionine sulphone (from methionine)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Valine	4	4	9	10	7	<1	5	9	4	6	7	9	7	2	6	10	10	9	.	8	9
Methionine sulphoxide	.	.	?	1	.	2	2	2	.	.	.	2	.	.	.	1	1	.	?	5	4
γ-Aminobutyric acid	<1	<1	<1	?	?	4	6	?	.	.	<1	.	.	.	.	<1	.	.	?	5	5
Lysine	2	3	6	9	5	6	7	6	2	4	6	8	6	3	6	10	10	7	2	>10	⩾10
Phenylalanine	.	<1	2	3	1	.	1	<1	.	.	1	1	<1	.	1	4	5	1	.	5	3
Leucines	4	5	8	10	6	.	7	8	<1	5	8	9	7	2	7	10	10	8	.	10	10
'Fast aminobutyric acid'	<1	<1	1	.	<1	.	.	1	<1	<1	<1	.	<1	.	.	<1	.	.	.	.	.
Proline	.	.	+	+	.	.	.	+	<+	<+	<+	+	<+	.	.	+	+	+	.	.	.
Arginine	3	4	5	7	3	.	3	5	3	4	6	6	6	2	5	8	8	6	.	9	7

* Many peptides also seen.
§ Many peptides and unknowns.
† Inferior vena cava.
‖ Many unknowns also; peptides have disappeared.
‡ Methylhistidine and unknown amino-acids also.

Summary of results given in Tables 1–3

Casein. Ingestion of this protein caused large rises in portal and jugular concentrations of amino-acids. The highest concentration of amino-acids was obtained in Exp. 5. With the exception of glutamic acid, the rise in individual amino-acids was consistent with what would be expected from the complete absorption of a casein hydrolysate (Figs. 8 and 9). It is remarkable that glutamic acid which occurs in casein to the extent of over 20% should not increase more in the portal blood during the digestion. As the two samples in Figs. 8 and 9 were matched as to total amounts of amino nitrogen, clearly the excess of glutamic acid in Fig. 9 has to be associated with a weakening of the spots due to the other amino-acids.

The vomit obtained in Exp. 4, 4 hr. after feeding the casein, showed a very large concentration of amino-acids and of peptides. The free amino nitrogen concentration could be roughly estimated as being about 20 times that of normal blood.

Casein hydrolysate (*Amigen*). As expected, ingestion of this was followed by large rises in portal amino-acids and only slightly smaller rises in the jugular blood. No appreciable synthesis of protein or of peptides by the intestinal mucous membrane could have occurred. The rate of absorption was only slightly faster than in the case of unhydrolyzed casein. The passage of this substance through the body can be seen best in the diagrams in Figs. 10–13. The original Amigen contains 'peptides' as can be seen more readily than on Fig. 10 by running chromatograms on larger amounts of Amigen or on concentrated fractions. The 'peptides' tend to concentrate in the urine, relative to the amounts of free amino-acids excreted (see also Christensen, Lynch & Powers, 1946). The figures also show well the invariable action of the kidneys in retaining preferentially the essential amino-acids and not so well the unessential. For instance, leucine is present in relatively smaller quantity in the urine than in the blood, glycine in relatively larger amounts.

Ground beef (*Hamburger*). Ingestion of this also resulted in large increases in portal amino-acids. The peak of digestion at 2·5 hr., however, corresponded to a lower concentration of amino-acids in the portal blood than was found after feeding equivalent amounts of any of the other proteins. There was evidence of a little bound amino nitrogen in the blood collected at this time. The peculiar composition of the 1 hr. specimen is also worthy of note. There was a rise in the concentration of some amino-acids, accompanied by a fall in the concentration of others, thus making an estimate of total amino nitrogen change much more difficult. If a change did occur in total amino nitrogen it could not have been appreciable.

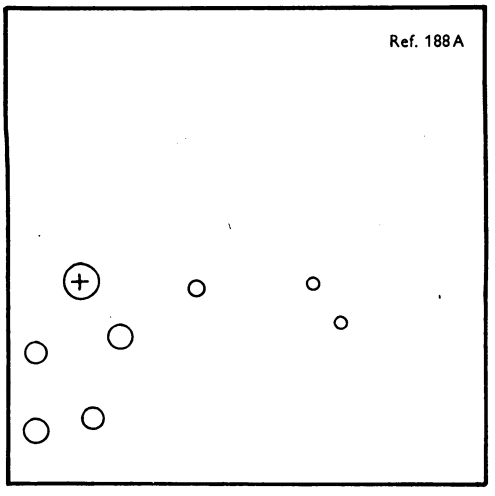

Fig. 14.

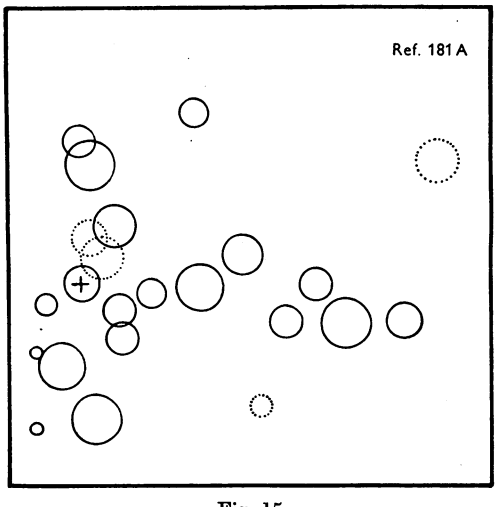

Fig. 15.

Figs. 14 and 15. Diagrams of the chromatograms from the urines of Exp. 13 (human-serum albumin feeding). The same conventions have been used as in Figs. 10–13. The three small spots above each other along the left-hand margin of Fig. 15, and the two corresponding ones of Fig. 14, represent unknown amino-acids; the middle one is in the position occupied by 'fast arginine', a basic amino-acid already found in other sources (Dent, 1948). The spot marked with a cross is believed to be due to methylhistidine. The material analyzed on each chromatogram was: Fig. 14, 25 μl. of the urine passed just before the experiment was begun; Fig. 15, 25 μl. of the urine secreted in the first 4 hr. of the experiment. Note the gross amino-aciduria and peptiduria in Fig. 15.

Human-serum albumin. The rise in amino-acid concentrations in the portal blood in Exp. 13 was the highest of any of these experiments (Fig. 6). Even at this concentration of digestion products, no

peptides were detected. There was, however, some bound amino nitrogen. The urine showed spectacular changes (Figs. 14 and 15). There was a gross amino-aciduria; at least four definite 'peptides' were present and the excretion of glutamic acid was phenomenally high. There were also some unknown amino-acids and on hydrolysis a large rise in the content of histidine and of β-alanine occurred. The large amount of β-alanine, which gave no colour reaction with *p*-dimethylaminobenzaldehyde, allowed of a definite distinction from citrulline. This extraordinary picture was exactly reproduced qualitatively, although in slightly less concentration, in the other experiment (Exp. 9) in which human-serum albumin was fed more slowly to a different dog.

Dog whole-plasma protein. Ingestion of this protein was not followed by any appreciable changes in the portal blood levels of amino-acids. The contrast between this result and those obtained with other proteins is definite and unambiguous. The best experiment technically was Exp. 6 because all the 100 g. of protein fed was retained, and the portal blood was followed for 8 hr. altogether. There seemed to be a slight but definite fall in plasma amino-acid concentration in the middle specimens. There was a slight rise in amino-acid concentration towards the end of Exp. 8. This was much less than in any of the experiments feeding other proteins. It may, however, be that this does represent slight digestion of the protein. In this experiment the gut was confirmed to be almost empty 5 hr. after the feeding, so there is no question of the protein having remained in the gut unchanged and unabsorbed.

DISCUSSION

The results obtained here show that large rises in the concentrations of many amino-acids occur in the portal blood of the partially gastrectomized dog after ingestion of casein, casein hydrolysate, ground beef and human-serum albumin. In marked contrast to this, however, in the three experiments in which dog whole-plasma protein was given, only relatively insignificant changes were found in the portal blood, although in one dog it was shown that the protein had disappeared from the gut at the end of the experiment.

In the group of experiments in which the heterologous proteins were given to the dogs, it has been shown that the blood amino-acids and other ninhydrin-reacting substances fall into three groups; those which rise and fall markedly and at about the same rates in their blood concentrations after the protein meal; those which vary less regularly tending if anything to rise slightly throughout the 5 hr. period, and those which are present in only small quantities and do not change appreciably during the experiment. The first group comprises the amino-acids commonly found in protein hydrolysates. This group is not completely accounted for, since hydroxyproline and tryptophan, which, present in small amounts in the proteins fed, would be expected to occur only in low concentrations, were never detected, and since methionine and cystine were not looked for systematically. Methionine sulphoxide, although not thought to occur in proteins, was detected and appeared also to belong to this group (Exps. 5 and 7). The second group comprises α- and γ-aminobutyric acids and perhaps glutamine. The third group comprises taurine, citrulline (and/or β-alanine) and the unidentified amino-acid 'fast aminobutyric acid'.

The amino-acids belonging to the first group are interpreted to represent the most important, possibly the only, compounds by which the original protein is transferred from the gut into the portal blood prior to its delivery to the liver and tissues. This view is based partly on the fact that these amino-acids are those which are liberated by hydrolysis of the protein in question. In one experiment (Exp. 5) in which casein was fed, it was further possible to show chromatographically that, with the exception of glutamic acid, the rise in concentration of each of the amino-acids corresponded roughly to what would be expected when the appropriate amount of the protein hydrolysate was added to the fasting blood (see Figs. 8 and 9).

Further suggestive evidence that the excess of amino-acids found in the portal blood represents the products of protein digestion and absorption is to be obtained by comparing the portal blood levels with those in systemic blood. Chromatographic analysis showed that in the casein experiment (Exp. 2) the portal blood had definitely higher concentrations of amino-acids than the systemic blood although there were no significant qualitative differences. The same result was obtained after feeding casein hydrolysate (Exp. 7, and Figs. 11 and 12) where there could be less doubt that absorption as amino-acids was taking place. These chromatographic results are supported by the more numerous and accurate quantitative analyses of Christensen (Addendum to this paper), who found that the total and free α-amino nitrogen was appreciably higher in portal than in systemic blood in all 1 and 2·5 hr. specimens, although they were sometimes approximately equal in the 5 hr. specimen when presumably the absorption from the gut was at an end.

The second group of amino-acids, which occur in the blood, are interpreted as being compounds released by the tissues after the further metabolism of the common amino-acids. This would account for the tendency to rise slowly in concentration during the 5 hr. period. There is some evidence that

α-aminobutyric acid is formed from methionine (Dent, 1947), presumably representing what is left behind after it has lost its methyl group and sulphur atom. γ-Aminobutyric acid is most likely to arise from the decarboxylation of glutamic acid although no direct evidence on this point has been obtained here or in the past except in experiments on putrefaction (Ackermann & Kutscher, 1910). It is difficult to draw conclusions from the behaviour of glutamine since this substance usually gives a distorted spot on the chromatograms. Moreover, the rough quantitative determinations of glutamine by the chromatographic method using the strength of the ninhydrin colour reaction do not give figures as high as have been reported for dog plasma by enzymic (Archibald, 1944) and by chemical (Hamilton, 1945) methods. The reasons for this discrepancy are not clear.

The amino-acids of the third group are believed to play no part in the processes occurring during protein digestion.

The objection that the blood amino-acid levels are too low to account for the observed transference of nitrogen from one part of the body to another can be disposed of by a consideration of the volume of blood flow in question. In a normal dog weighing 10 kg. the portal-plasma flow can be taken to be roughly about 200 ml./min. (Burton-Opitz, 1911; Macleod & Pearce, 1914; Soskin, Essex, Herrick & Mann, 1938; Lipscomb & Crandall, 1947). Even at this rate of flow a concentration of 4 mg. of amino nitrogen/100 ml. can transfer several grams of nitrogen in the 5 hr. span of an experiment. During digestion of a protein meal it is likely that a large increase in portal flow occurs which would be adequate to transfer all the nitrogen given in these experiments from the gut to the tissues in the form of amino-acids.

No evidence was obtained that peptides can be absorbed in any appreciable amounts since none was found in the blood by the various chromatographic methods tried, even under the circumstances induced by the partial gastrectomy operation, which caused rises in total free amino nitrogen concentration of up to six times normal (see Addendum). The chemical results of Christensen (Addendum) do, however, indicate that about 1 mg./100 ml. of 'bound α-amino nitrogen' is present in the blood and that this amount increases during the course of protein digestion. Similar rises after hydrolysis in the concentration of some amino-acids were also found by chromatography (Exp. 12). There is considerable doubt as to the nature of this bound nitrogen. It may be due to peptides, but could equally well be from amino-acids substituted, like the glycine in hippuric acid, on the α-amino nitrogen atom. There is indirect evidence for the existence of further examples of such acylated amino-acids. It is interesting that in some of the experiments recorded here (e.g. Exps. 7, 9 and 13) increases after hydrolysis in the amounts of free histidine and citrulline (and/or β-alanine) in the plasma were noted. In view of the definite identification of the latter as β-alanine in the urine in one case (Exp. 13) it may be that the substance increasing after hydrolysis is always β-alanine, and if so it would be very likely that they both arise from the presence of carnosine in the blood. Carnosine can be detected by paper chromatography (Dent, 1948), but may be missed in low concentrations. Although it is a true peptide it could not arise from simple hydrolysis of a protein. A liberation of carnosine from the tissues into the blood, coincident with a rapid uptake from the blood by the tissue cells of free amino-acids from the protein digestion, agrees with the views of Christensen, Streicher & Elbinger (1948). In guinea pigs amino-acids appear to compete with each other for the means by which they are concentrated by the cell, with the result that the ability of tissue cells to hold one amino-acid may decrease when other amino-acids are present in high concentrations in the blood. Such a rule might hold for a simple peptide like carnosine. Another reason for considering that the protein is not appreciably absorbed into the portal blood as peptides is based on the results of Exp. 4. In this experiment the intestinal contents vomited by the dog 4·5 hr. after giving the casein contained amino-acids and very high concentrations of peptides, most of the amino nitrogen being in peptide form. The chemical analysis also done by Christensen on this sample confirmed this result. However, the portal-blood samples obtained at 2·5 and 5 hr. and worked up and analyzed in exactly the same ways showed only the usual small amounts of bound amino nitrogen and no definite evidence of peptides. It would seem from this that the intestinal mucous membrane was acting as a very efficient barrier to the absorption of peptides. However, the finding of some 'peptides' in the urine when these were not detected in the blood (Exps. 7, 9 and 13; Figs. 13 and 15) does indicate the possibility of the absorption of small quantities. It was remarkable that the same four 'peptides' were found in the urine in the two experiments in which human-serum albumin was fed. The urinary concentration of blood peptides after the intravenous infusion of Amigen has been observed by Christensen *et al.* (1946).

With regard to the results found after ingestion of dog whole-plasma protein it must first be emphasized that all the evidence obtained here has been negative in character. The homologous protein disappeared from the gut and no trace of it was found in the portal or systemic blood by the same methods as were applied in the experiments with heterologous proteins. Several explanations of this

can be advanced. Technical errors in the chromatographic analysis are not considered likely in view of the very definite results and of the many control samples taken. Moreover, the entirely independent chemical analyses of Christensen (Addendum) have given the same results in the case of Exp. 6. We are left then with the following possibilities. First, that the protein is broken down to amino-acids in the gut, and that the intestinal mucous membrane resynthesizes them into plasma protein, in which form they are then taken up by the portal blood. Secondly, the protein may be absorbed into the portal blood as large peptides, which, if of high molecular weight, would not be detected by the methods used. Thirdly, the protein is absorbed largely in an intact or undigested form.

The possibility of absorption of homologous intact plasma protein should be considered seriously, particularly in the light of present views upon the mechanism of particulate fat absorption and upon the passage of plasma proteins through cellular membranes (Madden & Whipple, 1940). It is interesting too to remark on the peculiar gut reaction which always occurred when the plasma was given. The tendency to diarrhoea and vomiting, not related to the strong salt concentration of the plasma (Exp. 6), indicates that an abnormal state of affairs was induced in the intestine. Intact absorption of toxins and antitoxins from the gut in the adult animal can only occur after the mucous membrane has been damaged by simultaneous feeding of bile or of purgatives such as castor oil or aloes (Grasset, 1929). It was unfortunate that Exp. 10, in which denatured homologous plasma was fed, failed because of the exceptionally severe vomiting, for it would have been difficult to imagine that such denatured protein could be absorbed intact. A serious difficulty in accepting any theory of intact absorption concerns the expected action of the gut enzymes. A preliminary experiment (unpublished work) in which dog plasma was incubated in turn with gastric and mixed intestinal juices obtained from other dogs, and then analyzed on paper chromatograms has shown conclusively that complete breakdown to free amino-acids can result. If the experiments recorded here are to be reconciled with the theory of intact absorption it has to be assumed that this absorption takes place far more rapidly than the enzymic process or that the stimulus for secretion of the enzymes is lacking when homologous plasma is fed. A further objection exists, however, that cannot be readily disposed of. Clearly, if fed homologous plasma is absorbed intact then the nutritional result should be in every way identical with that obtained by giving the same amount intravenously; a fact which would be of possible clinical value. This, however, is not the case. Plasma protein given by vein to dogs in nitrogen equilibrium produces a definite nitrogen retention lasting some days, while the same dose by mouth, however, produces the full amount of extra nitrogen in the urine (Holman, Mahoney & Whipple, 1934). No increased urinary output of sugar occurs in phlorrhizinized dogs after giving plasma by vein, although there is an increase after giving it by mouth (Howland & Hawkins, 1938). Yuile & Hawkins (1941) later showed that a marked azotaemia occurred after feeding homologous plasma, good evidence that it was being metabolized. Similar differences in response to fed and injected plasma protein have been noted in human subjects (Forbes, Albright, Reifenstein, Bryant, Cox & Dempsey, 1948; Eckhardt, Lewis, Murphy, Batchelor & Davidson, 1948).

A possible explanation is that the plasma is associated with a nitrogen-retaining hormone, destroyed on passage through the liver and therefore only capable of exerting its action on the nitrogen balance when in the systemic, rather than in the portal, circulation. It is easier, however, to assume that the homologous protein is partly at least broken down in the gut before absorption, and that in this condition it cannot be resynthesized to the original protein. This would conform with modern views that the processes of synthesis and degradation of proteins are quite distinct and irreversible.

The fact that each of the dogs given homologous plasma had had a partial gastrectomy may be the explanation for the possible differences between these results and those of other workers.

The passage of intact homologous plasma protein through intestinal mucous membranes has long ago been demonstrated (Voit & Bauer, 1869; Heidenhain, 1894). It is also well known that homologous plasma protein disappears when placed in many serous cavities in the body, e.g. peritoneal or pericardial (Hamburger, 1895).

At the present time it is not possible to decide in favour of any of the above mechanisms of homologous protein absorption from the gut. Further work is in progress on this subject as most of the theories suggested are open to direct experimental trial.

SUMMARY

1. Dog portal blood removed by means of the London (1935) cannula has been examined by paper chromatography at intervals after feeding various proteins. Jugular blood was also examined in some dogs. A partial gastrectomy operation had been performed on most of the dogs as this was shown to result in more rapid disappearance of the protein from the gut.

2. Under these conditions the ingestion of heterologous proteins such as casein, casein hydrolysate (Amigen), ground beef and human-serum albumin,

resulted in large increases in the amino-acid concentration of the portal blood. The jugular blood showed quantitatively smaller but qualitatively similar changes.

3. After ingestion of casein the rises in the concentrations of individual amino-acids in the portal blood paralleled closely those which could be brought about by adding the appropriate amount of casein hydrolysate to the fasting blood in an *in vitro* experiment. Glutamic acid, however, behaved in an exceptional manner for it was present in the portal blood to a much smaller extent than in the artificial mixture.

4. Peptides were not definitely found in the portal blood in any instance. Some evidence was obtained for the occasional presence of bound amino nitrogen of unknown identity. In some cases this was likely to have been in the form of carnosine.

5. After ingestion of human-serum albumin the urine contained excessive quantities of many amino-acids, especially of glutamic acid. There were also several 'peptides'. Similar but less prominent changes in the urine were found after ingestion of Amigen.

6. The blood amino-acids appeared to belong to three types: those which rose and fell characteristically after the protein meal, those which varied less regularly, tending if anything to rise slightly throughout the 5 hr. period, and those which did not change appreciably during the experiment.

7. Dog whole-plasma protein when given to the dogs by mouth did not cause any significant rise in portal amino-acid concentration, and neither peptides nor bound amino nitrogen were found.

8. It is suggested that the homologous plasma protein was absorbed either intact or as large fragments, and that the heterologous proteins may have been largely if not entirely absorbed as free amino-acids.

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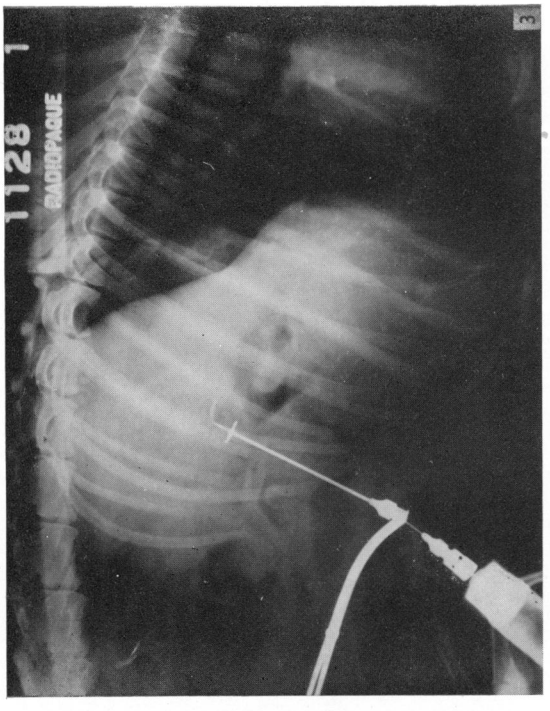

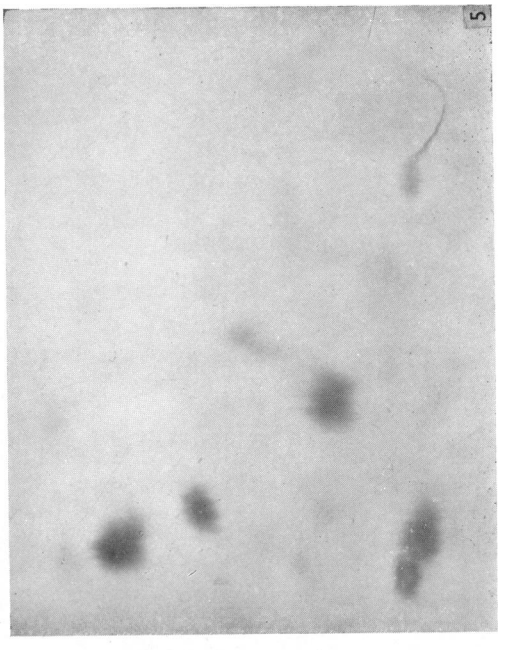

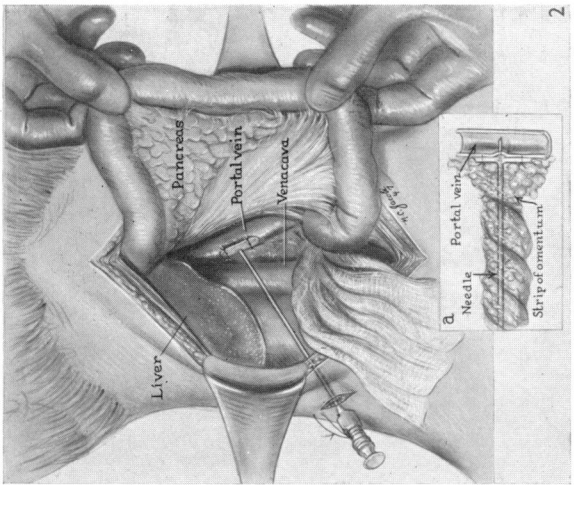

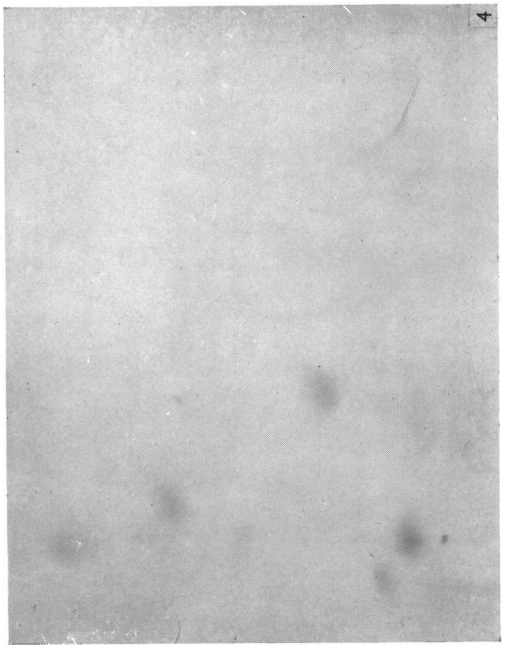

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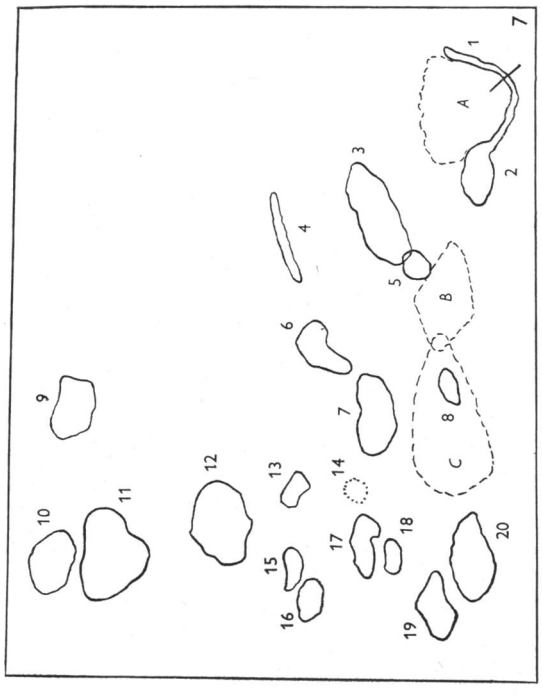

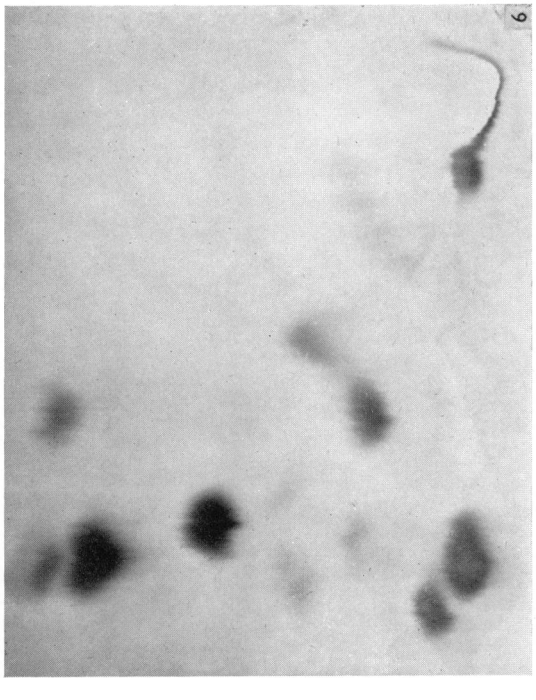

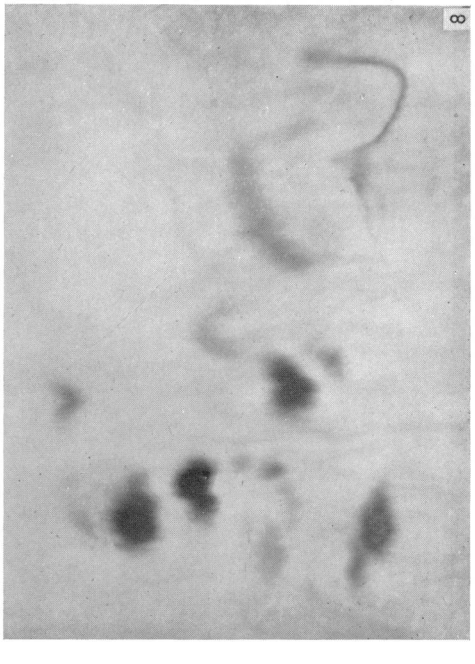

C. E. DENT AND J. A. SCHILLING—STUDIES ON THE ABSORPTION OF PROTEINS: THE AMINO-ACID PATTERN IN THE PORTAL BLOOD

EXPLANATION OF PLATES 4 AND 5

PLATE 4

Fig. 2. Drawing showing the London cannula sutured in its final position to the portal vein. A strip of omentum is wrapped round the cannula, as shown in the inset, before the abdomen is finally closed. To withdraw portal blood the trocar is removed and a long needle inserted through the cannula until the wall of the vein is pierced.

Fig. 3. X-ray photograph of dog with cannula in position during an injection of thorotrast into the portal vein (Exp. 5). The branches of the portal vein can be seen weakly outlined. This confirmed that true portal blood samples had been taken in the experiment.

Fig. 4. Photograph of chromatogram from a typical sample of fasting portal blood (Exp. 13). (In this and subsequent photographs, standard exposures and routine of development have been imposed.)

Fig. 5. Photograph of chromatogram from the portal blood 2·5 hr. after feeding 500 g. of ground beef (Exp. 12).

PLATE 5

Fig. 6. Photograph of chromatogram from the portal blood 2·5 hr. after feeding 100 g. of human albumin (Exp. 13). This shows the highest concentration of amino-acids seen in any of these experiments. It should be compared with fasting blood from the same experiment seen in Fig. 4.

Fig. 7. Key to Fig. 6. 1 = aspartic acid, 2 = glutamic acid, 3 = serine, 4 = taurine, 5 = glycine, 6 = threonine, 7 = alanine, 8 = glutamine, 9 = tyrosine, 10 = phenylalanine, 11 = leucines, 12 = valine, 13 = α-amino-*n*-butyric acid, 14 = histidine (this does not appear in Fig. 6 but can be seen in Figs. 5, 8 and 9), 15 = 'fast-aminobutyric acid', 16 = proline, 17 = methionine sulphoxide, 18 = γ-amino-butyric acid, 19 = arginine, 20 = lysine. In addition three unidentified substances, *A*, *B* and *C*, are shown, which appear, where outlined, as yellow areas. Substance *A* causes the serious distortion of aspartic and glutamic acids which are pushed apart in the upper region and caused to run into one another below. The oblique line shows the approximate dividing line between the two amino-acids as determined by the use of markers. *B* and *C* usually overlap slightly, as shown. *B* causes the least trouble in practice; *C*, however, may overlie glutamine or push it slightly up or down. Serine and taurine and, to a less extent, threonine are also pulled into long streaks, presumably while they are moving with the yellow substance during the phenol run.

Fig. 8. Photograph of chromatogram from the portal blood 2·5 hr. after feeding 100 g. of casein (Exp. 5). This should be compared closely with Fig. 9. See text for further discussion.

Fig. 9. Photograph of chromatogram from fasting portal blood (Exp. 5) with enough acid-hydrolyzed casein added to it to match the total amino-nitrogen level of the sample shown in Fig. 8.

Addendum. Conjugated Amino-acids in Portal Plasma of Dogs after Protein Feeding

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The best available evidence for the absorption of important quantities of peptides during protein digestion came from the laboratory of Dr E. S. London from experiments upon dogs having cannulae affixed to various blood vessels (for references, see Christensen, Decker, Lynch, Mackenzie & Powers, 1947). The deficiencies in the analytical procedures used in this field have been discussed (Christensen *et al.* 1947). A re-examination of the question in dogs with cannulated portal veins has been made possible through the kindness of Dr C. E. Dent in supplying us with ultrafiltrates of plasma obtained from dogs in experiments described in the preceding paper (Dent & Schilling, 1949). Analysis of these samples for α-amino nitrogen by the manometric ninhydrin procedure, before and after acid hydrolysis, showed measurable increases in the amino-acid conjugates accompanied by very large rises in free amino-acid concentrations.

EXPERIMENTAL

α-Amino N was determined in replicate by the manometric ninhydrin method at pH 2·5 (Hamilton & Van Slyke, 1943) upon samples of plasma ultrafiltrate before and after

hydrolysis by acid (Christensen & Lynch, 1946). In the case of dog 14, glutamine was also determined by the loss of α-amino N upon prolonged heating (Hamilton, 1945). In this experiment the free and bound α-amino N were determined at pH 4·7 (not pH 2·5). Glycine was determined by the formaldehyde released by ninhydrin (Alexander, Landwehr & Seligman, 1945).

DISCUSSION

Significant increases in the amino-acid conjugates of plasma were observed after the feeding of casein, ground beef and human-serum albumin (Table 1). These increases were far smaller, however, than those of free amino-acids. Furthermore, large and consistent portal-jugular concentration differences were found in the free amino-acids but not in the conjugates, suggesting that the latter were perhaps entering the blood from the digestive tract much more slowly than were the free amino-acids. As a result of the partial gastrectomy, and the large size of the protein feeding, the ratio of the amount of protein to the amount of digestive enzymes present in the intestine was undoubtedly unusually large. This might be expected to favour the absorption of intermediate degradation products of the proteins. During digestion the intestine contained large quantities of peptides as indicated by the analysis of the vomitus of dog 4, obtained 4·5 hr. after the test meal had been given. Of the ultrafilterable amino-acid nitrogen present, 84% was in peptide form, 16% free (Dent & Schilling, 1949).

The conjugates found in plasma during protein digestion in these experiments were not necessarily fragments of the proteins fed, since the feeding of L-glutamic acid to dogs (Table 1) or of glycine to humans (Christensen, Cooper, Johnson & Lynch, 1947) produced increases in the conjugates of plasma.

A decrease in the glycine concentration of plasma, as well as a net decrease in the non-glycine, non-glutamine amino-acids, was observed during glutamate absorption (Table 1). Dent has observed decreases in other amino-acids in the same samples by paper chromatography. This chromatogram on 125 μl. of the 1 hr. portal-plasma ultrafiltrate showed, apart from the very large quantities of glutamic acid, only a slightly raised concentration of alanine, and just detectable traces of proline and of arginine. All the other amino-acids were undetectable (Dent, private communication). These observations, together with similar observations in guinea pigs, will be discussed elsewhere (Christensen, Streicher & Elbinger, 1948).

Table 1. *Amino-acids of portal and jugular plasma after feeding of protein and of glutamic acid*

(The values are in mg./100 ml. of ultrafiltrate.)

Dog. no.	Material fed	Type of α-NH_2-N	0 hr.		1 hr.		2·5 hr.		5 hr.	
			Portal	Jugular	Portal	Jugular	Portal	Jugular	Portal	Jugular
4	Casein (100 g.)	Total	5·17	5·57	11·61	10·47	14·74	12·70	6·03	8·68
		Free	4·56	5·02	10·11	9·37	14·46	11·59	5·58	8·24
		Bound	0·61	0·55	1·5	1·1	0·3	1·1	0·45	0·44
5	Casein (100 g.)	Total	4·84	4·97	16·5	13·0	18·9	12·2	10·4	11·56
		Free	4·41	3·88	13·4	10·80	16·3	9·30	10·2	9·06
		Bound	0·43	1·1	3·1	2·2	2·6	2·9	0·2	2·5
6	Canine plasma protein (100 g.)	Free	4·10	3·53	3·94	3·06	2·69	3·18	3·24*	3·06*
12	Ground beef (500 g.)	Total	6·6	5·4	6·73	5·6	16·3	10·9	14·90	8·88
		Free	5·84	4·64	5·84	4·62	14·8	—	13·75	8·32
		Bound	0·8	0·8	0·9	1·0	1·5	—	1·2	0·6
13	Human-serum albumin (100 g.)	Total	5·10	—	17·1	—	23·8	19·1	10·13	8·33
		Free	4·26	3·83	14·4	10·15	21·9	16·9	9·00	7·43
		Bound†	0·8	—	2·7	—	1·9	2·2	1·1	0·9
14	L-Glutamic acid (as glutamate) (3 g./kg.)	Glutamine	1·10	1·21	1·59	1·59	0·98	1·10	0·71	0·99
		Glycine	0·20	0·19	0·24	0·22	0·15	0·13	0·17	0·13
		Non-glycine, non-glutamine	2·69	2·79	9·99	8·08	4·46	3·80	2·24	2·02
		Bound	0·92	0·59	—	0·47	0·71	0·81	1·38	1·12

* These two samples were taken at 8 hr.

† These values include no appreciable amount of acetyltryptophan. The urine excreted during this test contained 342 mg./100 ml. of free α-amino N, which was 21 times the pre-test concentration.

SUMMARY

1. During the digestion of several protein meals by partially gastrectomized dogs, increases occurred in the concentrations of conjugated amino-acids of portal and jugular plasma.

2. The results indicate that at most a minor part of the protein was absorbed in peptide form. The plasma conjugates were not necessarily fragments of the proteins fed since increases in plasma conjugates were also produced by feeding L-glutamic acid.

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The Metabolism of Sulphonamides

6. THE FATE OF SOME N^4-*n*-ACYL DERIVATIVES OF AMBAMIDE (MARFANIL) AND THE SULPHONE, V 335, IN THE RABBIT

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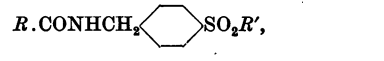
We have shown (Hartles & Williams, 1947) that the drugs ambamide (Marfanil) and V 335 lack systemic antibacterial activity because they are rapidly deaminized and oxidized to inactive metabolites. We suggested that the marked *in vitro* activity of these drugs might be preserved *in vivo* if the biologically labile $-CH_2NH_2$ group could be protected by a group which is removed in the body at a rate which allows therapeutic concentrations of the free drugs to be attained. Acetylambamide (*p*-acetamidomethylbenzenesulphonamide), which has no antibacterial activity (Klarer, 1941), is however excreted unchanged (Hartles & Williams, 1947). The acetyl group certainly protects the $-CH_2NH_2$ group against deamination and oxidation, but is itself resistant to hydrolysis in the body. It does not follow, however, that, if the acetyl derivatives are resistant to hydrolysis *in vivo*, then other acylamino derivatives are also resistant. We have therefore synthesized and studied the fate of a number of *n*-acyl derivatives of the type



where $R.CO$ is a *n*-acyl group, and R' is $-NH_2$ in ambamide derivatives and $-CH_3$ in V 335 derivatives.

Not only have we searched for possible therapeutic agents among these compounds, but we have also noted a relationship between the chain length and the degree of hydrolysis of the acylamino group. The extent of hydrolysis was assessed from the output in the urine of either *p*-carboxybenzenesulphonamide or *p*-methylsulphonylbenzoic acid and unchanged acyl derivatives, the reactions assumed to take place being

(*a*) $R.CO.NH.CH_2.C_6H_4.SO_2NH_2 \rightarrow R.COOH + NH_2.CH_2.C_6H_4.SO_2NH_2 \rightarrow COOH.C_6H_4.SO_2NH_2$ for ambamide derivatives and

(*b*) $R.CO.NH.CH_2.C_6H_4.SO_2CH_3 \rightarrow R.COOH + NH_2.CH_2.C_6H_4.SO_2CH_3 \rightarrow COOH.C_6H_4.SO_2CH_3$ for V 335 derivatives.

EXPERIMENTAL

Materials and methods

Synthesis of n-*acyl derivatives of ambamide and V* 335. With the exception of acetylambamide (Klarer, 1941; Bergeim & Braker, 1944) and acetyl V 335 (Jensen, Schmith, Brandt, Lauritson & Hanson, 1944) all the compounds now described are new and were prepared by the same general method.

p-Aminomethylbenzenesulphonamide, m.p. 153° (ambamide base), or *p*-aminomethylphenyl methyl sulphone, m.p. 85° (V 335 base), was heated with a molecular equivalent of the fatty acid for 30 min. at 150–160°. The mixture was cooled, broken up and recrystallized from water (for formyl, propionyl and butyryl derivatives) or from ethanol (for C_6–C_{18} acyl derivatives). The analytical data and melting points for these compounds are given in Table 1. All the compounds appeared white or colourless and the yields were 50–60% of theory.