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General Discussion

Professor J. R. Learmonth (Department of Surgery, University New Buildings, Edinburgh): With regard to Dr. Cuthbertson's paper, it seems to me that there would be an opportunity for studying the dispersal of nitrogen from muscle, in nerve injuries which involve a nerve of the upper limb, particularly the musculo-spiral nerve whose division is followed by a rapid reduction in bulk of the extensor-supinator group of muscles, not contributed to by disuse from confinement to bed. I wonder also whether some information about the possible importance, use and fate of various amino-acid groups could be obtained by the use of tagged ions.

There are one or two clinical points that occur to me. It seemed to me that at the beginning of the war, with the dietary restrictions envisaged, there might be some difficulty in surgical practice with regard to the healing of wounds, and I have kept a record of the time spent in hospital after various major operations. The time spent in hospital has not been affected by war time diets.

There is one point with regard to the disruption of wounds; a great many factors are apparent to the clinician which are possibly not apparent to other workers in studies of this sort. In the first place, in the study of the disruption of a wound, the normal tensile strength of the tissue which is sutured should form an integral part of the preliminary consideration for the work, as well as the tensile strength of the suture material, which should not exceed the tensile strength of the tissue to be sutured. The type of suture employed should always be taken into consideration because it is important to know the effect of knots on tensile strength; a mattress suture, for instance, has a different tensile strength from a figure-of-eight suture.

In human work we are faced with a further difficulty that certain people seem to be peculiarly susceptible to certain forms of suture material. Some patients are "anaphylactic" to catgut, and wounds may break down when closed with this material. The materials used to harden sutures, such as chromic and tannic acids, may have an effect on the wound, and they vary from brand to brand. That ought always to be accurately assessed beforehand.

An interesting point is that if an abdominal wound bursts open, the second suture of the wound is usually attended by secure healing. There

are one or two points with regard to individual wounds. One sees in certain patients wounds behaving quite differently in different parts of the body. I remember, for instance, a boy whose primary wounds healed quite easily, whereas a wound made behind the knee healed with much deformity.

I wonder whether any work has been done on the effect of different types of bacteria on the actual nature of the process of healing and repair; whether some bacteria interfere with the process of repair by, for example, fibroblasts, I do not know. It seems to me that in experiments on animals in which it is impossible to secure sterility of the wound this factor should be taken into consideration. Is there any relation between the amount of active phosphatase in the wound, and the bacteria contaminating it? Could bacteria themselves affect the amount of phosphatase in a wound? We know that certain chemicals, *e.g.*, sulphonamides, interfere with the metabolism of certain bacteria, such as streptococci. May certain bacteria influence in the same specific way the metabolism of certain types of cells?

Dr. F. Bergel (Roche Products, Ltd., Welwyn Garden City, Herts.): I should like to draw attention to the work of Professor Campbell and Dr. Cook on the effect of ascorbic acid on the healing of tooth extraction wounds. These workers showed that, in the same patient, the healing process was more rapid after the administration of vitamin C than before (Campbell and Cook, 1945).

REFERENCE

Campbell, H. G. and Cook, R. P. (1945). *Proc. Nutr. Soc.* 3, 89.

Dr. J. F. Danielli (School of Biochemistry, Cambridge): How far were the results obtained invalidated by the effect of the first extraction on the second, *i.e.*, does the first injury affect the healing of the second injury?

Dr. S. K. Kon (National Institute for Research in Dairying, University of Reading): Did Dr. Cuthbertson and Mr. Croft find any change in the faecal excretion of nitrogen in animals or man after exposure to trauma? Am I correct in assuming that the negative nitrogen balances shown by Dr. Cuthbertson would prove even more negative if the faecal loss of nitrogen were considered? I should like to ask Dr. Cuthbertson also whether proteins differ in their effect on nitrogen loss in trauma according to their biological value.

Professor J. R. Marrack (London Hospital, Whitechapel, London, E.1): Was healing accelerated or time of convalescence shortened in the patients who were given high protein diets and in the rats which received methionine?

Dr. W. R. G. Atkins (Marine Biological Laboratory, Citadel Hill, Plymouth): Alizarin, used for locating calcium deposits, is an indicator giving a pink colour in alkaline solution which fades in acid. Bone sections mounted in Canada balsam may appear therefore to be without calcium. The molybdenum blue reaction proceeds in acid solution, detects phosphate and so locates calcified deposits.

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The records of the good results obtained with orange juice in certain surgical cases are of much interest. The examination of the vitamin C reserves of 1200 soldiers at home, which I carried out under the orders of the Director of Hygiene, showed that the general condition was satisfactory. This is in agreement with the statement of our Chairman that the time spent in hospital after surgical operations has not increased in war time.

Dr. C. F. Brockington (Public Health Department, Shire Hall, Warwick): Were any liver changes observed in the burned animals? Such changes might be expected to occur if it is true that methionine deficiency is a cause of liver damage.

Dr. T. Moore (Dunn Nutritional Laboratory, Cambridge): Has choline been tried in burns instead of methionine?

Mr. A. L. Bacharach (Glaxo Laboratories, Ltd., Greenford, Middlesex): I should like to know whether the methionine used at Oxford in the experiments described by Mr. Croft was the *dl*-mixture, and, if so, whether there is any information as to differences shown by the *d*- and *l*-forms in their effects on nitrogen balance. Biochemically speaking one of the most interesting questions raised in this discussion has been the site and mode of action of ascorbic acid in influencing the formation of connective tissue and thereby of phosphatase. Has any work been done on the direct injection of ascorbic acid into wounds in muscle or bone? Although some of the doses of ascorbic acid used in work on the healing of experimental wounds in animals were admittedly high, and although a clear optimum dose has been established below the highest dose used, it might still be of interest to use even higher doses, in order to make certain that a second "peak" has not been missed.

Dr. E. Kodicek (Dunn Nutritional Laboratory, Cambridge): We gave guineapigs amounts of ascorbic acid up to 10 mg. daily, but with more than 5 mg. neither histologically nor clinically could we find any difference.

Captain J. A. F. Stevenson, R.C.A.M.C. (A.M.D.5, D.M.S., C.M.H.Q., C.A.O.): I would like to ask Dr. Cuthbertson two or three questions. For the past few years Dr. J. S. L. Browne of the McGill University Clinic has been studying the phenomenon described by Dr. Cuthbertson this morning. I was associated with Dr. V. Schenker in carrying out this study under the direction of Dr. Browne. For the past year it has been my duty to help in the practical application of the results of these studies in Canadian Military Hospitals.

In my opinion the work done at the McGill University Clinic, and that which has been done in the United States on this problem, agree in general with the findings of Dr. Cuthbertson. However, I would like to ask Dr. Cuthbertson why he emphasizes the distinction Hunter made between trauma and disease. Our group has done nitrogen balance studies on a few acute diseases such as influenza, colds and conjunctivitis, and we have found that a similar protein catabolic reaction occurs at their onset. Shaffer and Coleman's (1909) studies of typhoid fever indicate that a similar catabolic reaction probably occurs there too. In view of these findings we have considered the hypothesis that this protein

catabolic reaction is a general response of the previously healthy, well nourished organism to damage.

I should like to ask Dr. Cuthbertson also if he has carried on any of his studies of nitrogen balance after fracture for a sufficient length of time after the damage, to ascertain what happens after the remission of the protein catabolic response. Dr. Browne, Dr. Schenker and I did this in a series of burns and fractures. We found that the protein catabolic period which occurs on damage was followed by a protein anabolic period in which the urinary excretion of nitrogen is lower than it would be in health on the same intake, particularly if the intake is high. At this time the body is apparently trying to replenish the protein tissue it has used up earlier. We feel that this is very important in curative medicine. While we do not know the advantages or disadvantages of very large intakes at the height of the protein catabolic period, we do know that high intakes, 150 g. of protein and 3500 to 4000 Calories a day, are very beneficial in the anabolic period, producing a marked positive nitrogen balance, a rapid replenishment of tissue, and a convalescence improved and shortened both subjectively and objectively. However, this protein anabolic tendency has very little chance to show itself on the intakes achieved in hospitals at present. There are so few hospital patients receiving optimum intakes that the disadvantages of the standards of food requirements accepted at present for convalescence do not stand out.

In the study at McGill we found, like Dr. Cuthbertson with his protein depleted rats, that patients previously ill and malnourished did not show nearly as marked a rise in nitrogen excretion, after operation or other damage, as did the healthy, well nourished individual. As to what is commonly called disuse atrophy, it is surprising how much less this occurs in patients kept on high food intakes.

REFERENCE

Shaffer, P. A. and Coleman, W. (1909). *Arch. intern. Med.* 4, 538.

Dr. J. Pemberton (Royal Hospital, Sheffield): I am at present taking part in an experiment on human volunteers who are receiving a diet deficient in vitamin C. We have encountered several difficulties in making standardized experimental wounds and in testing their strength. A linear incision 3 cm. long is now made in the lateral aspect of the thigh down to, but not including, the *fascia lata*. This is closed with 3 interrupted waxed silk sutures which are removed on the 5th day. Ten days, or in some cases 21 days, after the incision the whole wound is excised and the tensile strength of the middle centimetre tested. This method seems to yield comparable results. Attempts to estimate the tensile strength of the wound *in situ* were abandoned owing to the difficulty of confining the tension applied to the wounded tissues alone. Wounds excised after 10 days did not show much difference between control and experimental subjects; perhaps the time was too short to provide good contrast.

I have been impressed today with the results of biochemical research on nitrogen loss in burned animals and on the administration of vitamin C to animals after bone fracture. I think that some of this work could have

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been done with human beings and that it would have yielded results more quickly.

Dr. J. Waterlow (Medical Research Council Human Nutrition Research Unit, National Hospital, Queen Square, London, W.C.1): As Dr. Pemberton says the difference in tensile strength of wounds between normal and deficient subjects might be more marked at a later stage of healing. Dr. Fell kindly allowed me to see her slides from guineapigs and, unless I have misinterpreted them, histological differences between normal and deficient animals were much less marked at an early stage, after say 10 days, than later, after 21 to 28 days. This would fit in with the findings reported by Dr. Pemberton. On the other hand, Dr. Bourne described differences in tensile strength in guineapigs' wounds excised as early as after 7 days. It would be interesting to know the histological findings in Dr. Bourne's experiments, so that one could know what was the structure which, by its presence or absence, caused the difference in strength.

Dr. A. H. Hunt (St. Bartholomew's Hospital, West Smithfield, London, E.C.1): The question of the cellular element in wound healing has not been mentioned today. I observed in my experimental work that lack of vitamin C leads to deficient maturation of the fibroblasts.

Dr. H. B. Fell replied:

To Dr. Hunt: I have observed the same thing. The cells were much more embryonic in appearance in the scars of the deficient than of the normal animals.

To Professor Learmonth: Professor Learmonth asked about the possible effect of infection on the phosphatase content of wounds. We have not made a bacteriological investigation of wounds in our guineapigs but I can say that there have been a few wounds in both normal and deficient animals which were fairly obviously infected. The pus in both cases gave a very strong phosphatase reaction owing to the large numbers of polymorphonuclear leucocytes.

Dr. D. P. Cuthbertson replied:

To Professor Learmonth: Labelled elements should prove of great value in the study of the metabolic effects of injuries. Owing to the war this country has much leeway to make up in this direction. I am very glad to know that steps are now being taken to put the matter right.

I agree with the suggestion that immobilization, particularly that conditioned by pain, induces a more rapid wasting and, in consequence, loss of nitrogen, but I do not think that in the early days after injury this would be the main cause of the catabolic loss of protein. In this connexion it would certainly be interesting to study injuries involving the musculo-spiral nerve.

To Dr. Kon: In my experience the faecal nitrogen was not much affected by injury or the use of high protein diets. With human beings it has been my aim to supply protein of the highest possible biological value and I am, therefore, unable to say what would be the effect of giving protein of lower quality.

To Professor Marrack: It has not been possible to correlate clinical improvement with high protein intake as the patients varied greatly and were not observed for a sufficiently long time.

To Dr. Brockington: We meant, but were unable, because of lack of time, to examine the livers of our experimental animals.

To Captain Stevenson: The catabolic response to injury is probably in part a protective reaction and in the words of John Hunter produces both "the disposition and the means of cure".

We have not done nitrogen balance studies for longer than a month after injury so that I cannot confirm from personal experience the marked anabolic phase in the later stages of recovery mentioned by Captain Stevenson, but I have no doubt that it exists.

Mr. P. B. Croft replied:

To Dr. Kon: In our experiments upon rats, we did not find any significant change in the faecal nitrogen of burned rats compared with that of control groups of animals. Thus, it did not appear that there was any loss of nitrogen in the faeces comparable with that in the urine. The supplement of 1 per cent. of methionine also caused no increase in faecal nitrogen above that of the animals on the basal diet.

To Professor Marrack: We were not able to make any definite observations about clinical improvement and healing of the animals receiving diets supplemented with protein and methionine. Our metabolic experiments have been continued only for ten days after the burn, a time insufficient to be sure of the subsequent clinical progress of the animal.

To Dr. Brockington and Dr. Moore: We have so far no definite information about the extent of any possible liver damage in the burned animals. I agree that choline should be tried in this connexion. We are actually doing experiments with choline in Oxford at the present time, but they are not sufficiently advanced for me to be able to give a definite answer as to the result.

To Mr. Bacharach: We used *dl*-methionine in all our experiments, the synthetic amino-acid made for us by the Ministry of Supply. We have not had the natural isomer available in sufficient amounts to use it in this work.

To Dr. Pemberton: I do not agree with the suggestion that the kind of work reported by Dr. Cuthbertson and myself has been carried out too much in the laboratory and with insufficient attention to clinical trials. We have been impressed greatly by the difficulty of obtaining satisfactory controls even under ideal laboratory conditions, and I feel that it would only be possible to get good results on human patients by applying to them methods evolved in animal experiments when the problems likely to arise in human work had been investigated. We are, of course, hoping to be able to try methionine on human subjects suffering from severe burns.

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