the rat, but diazepam will cause an increase in contraction height by a direct effect upon the muscle.—We are, etc.,

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REFERENCE

- ¹ Hunter, A. R. *British Journal of Anaesthesia*, 1967, **39**, 633.

Early Breast Cancer

SIR,—In his article on the treatment of early breast cancer Mr. J. I. Burn (23 May, p. 467) comments on the administrative disadvantages of "multicentre" clinical trials, quotes Handley¹ as saying that a controlled trial should be carried out in a single unit by a single team, and suggests that if Deeley's system² of paired randomization is adopted "large numbers of patients may not be necessary." We feel that these remarks, with their implied discouragement of multicentre participation in clinical trials, are unfortunate at a time when the idea of widespread collaboration is at last gaining ground. It may also mislead people into embarking again on trials where the likely rate of patient intake will make the effort of very doubtful value.

Too few patients in a trial are a disadvantage for two reasons. Firstly, the randomization procedure, although eliminating obvious bias, may not result in the final treatment groups consisting of closely comparable clinical material. It may not therefore be possible to draw any valid conclusions from the trial. This is the problem dealt with by Deeley,² and his system of paired randomization is designed to give comparable groups even with small numbers in a trial.

The second disadvantage of small numbers, which is not overcome by Deeley's procedure, arises from the fact that if the difference we are looking for between two treatments is small (and it seems likely that this will be so in the cancer field), then it is not possible to demonstrate such a difference convincingly with small numbers. To give a simple example: if 100 patients are treated in each of two treatment groups and 60 survive in one compared with 50 surviving in the other then this difference is not statistically significant at the usually accepted 5% level, however carefully we may have designed our trial. But if there are 1,000 patients in each group, and 600 are cured by one treatment while only 500 are cured by the other, then a highly significant difference between the two treatments will have been demonstrated, although the percentages cured are still the same as in the smaller trial.

Collaboration between a number of centres therefore appears to us to be the only way of obtaining in a reasonably short time the numbers of patients required to make useful comparisons of cancer treatments possible. We have found great enthusiasm for this collaboration among surgeons working away from the major centres, and this enthusiasm, the surgeons' skill, and the numbers that they treat must not be lightly abandoned. The administrative difficulties of a multicentre trial are not insurmountable, and Burn's apparent prefer-

ence for the small trial in a single centre is, in our view, misguided. To be fair to the authors he quotes in his support it should also be noted that Deeley² makes no claim that pairing will reduce the numbers required for statistical significance, and Handley¹ advocated a trial "in the same unit by the same team" as being preferable to uncontrolled comparisons between different series each treated in different centres. He did not discuss at all the relative merits of single centre as compared with multicentre trials.—We are, etc.,

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- ¹ Handley, R. S., *Cancer*, 1969, **24**, 1270.
² Deeley, T. J., *Methods of Information in Medicine*, 1966, **5**, 100.

Purification of Ampicillin

SIR,—According to a recent article concerning the incidence of rash and the purification of ampicillin (Penbritin), Dr. E. T. Knudsen and others (21 February, p. 469) isolated protein from their ampicillin preparations and found it to be immunogenic. We have examined eight different lots of ampicillin produced by Bristol Laboratories U.S.A. (Polycillin) for protein, and found none to be present.

Dr. Knudsen and colleagues cite no procedure used for the isolation of protein impurity and no procedure for identifying their contaminant as protein. We isolated macromolecular material by a preliminary sterile molecular sieving of 300-g. batches of ampicillin through Sephadex G25. The macromolecular fractions were then subjected to dialysis through cellophane dialysis tubing against a constant flow of water. The retentate obtained was hydrolyzed with hydrochloric acid in a sealed tube and analyzed with a Technicon Autoanalyzer for lysine, proline, and histidine as indicators of protein content. With this technique an amidase enzyme, when introduced as a contaminant in ampicillin, would be detected at a concentration of one $\mu\text{g./g.}$ of ampicillin. The eight lots of Bristol ampicillin tested showed no evidence of protein content. Traditionally ampicillin has been produced by utilizing 6-aminopenicillanic acid obtained by cleavage of penicillin G with a bacterial enzyme, thus potentially introducing a source of protein contamination. We wish to report that presently all of our 6-aminopenicillanic acid is obtained through a completely chemical process of splitting a natural penicillin to yield 6-aminopenicillanic acid,¹ thus completely eliminating any possibility of bacterial enzyme as a source of protein contamination of the final product.

Immunogenic response to sensitizing protein has been considered to be a more critical test than chemical analysis. The immunogenic liability of non-protein macromolecular material isolated from our ampicillin was tested following procedures similar to those described by Dr. Knudsen and others. We found no evidence of an immune response (penicilloyl-specific antibody production) using either complete

or incomplete Freund's adjuvant. Interpretation of the results presented by Dr. Knudsen and others in Table I is open to some question in view of the report of Batchelor and Dewdney² describing the rapid penicilloylation of the mycobacteria of Freund's adjuvant. Complete details of our work will be reported elsewhere (O. B. Fardig, *et al.*, in press).—We are, etc.,

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REFERENCES

- ¹ Weissenburger, H. W. O., and van der Hoven, M. R., U.S. Patent 3,499,909, 1970. Washington, U.S. Patent Office.
² Batchelor, F. R., and Dewdney, J. M., *Proceedings of the Royal Society of Medicine*, 1968, **61**, 897.

Pancreatic Enzyme Therapy

SIR,—We have read with interest the Today's Drugs article on pancreatic extracts (18 April, p. 161), and although we agree with several points made in the article, such as the erratic nature of the release of the drug from enteric coated tablets and the advisability of administering pancreatic extracts together with food, there are several points on which our opinions diverge.

Figure illustrates the treatment of a patient with pancreatic enzyme deficiency with pancreatic replacement.

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Such points include the frequently unimpressive nature of the results of pancreatic enzyme therapy; also the suggestion that treatment with pancreatic extracts is not essential, as fat absorption can proceed without lipase and can be enhanced by increasing the dietary intake of fat. Though this indeed can occur, it is usually