published.³ The failure to demonstrate higher levels of this activity in our mouse test—injecting the animals with chronic idiopathic thrombocytopenic purpura plasma—needs to be further elucidated.

It was a pity that Dr. Penington did not classify his positive cases. Although deficiency in feed-back inhibition could be considered in thrombocytopenia, my colleagues demonstrated that the giant-cell content of the protected areas of x-ray treated mice can be increased with "positive" plasma (received after irradiation) even in animals adjusted to normal platelet levels by means of syngeneic thrombocyte transfusions.⁴

Comparing the thrombopoietic effects of from 0.4 to 2.0 ml. subcutaneously administered plasma samples using his isotope bioassay method Dr. Penington states that "the results presented are the first in which a dose-response relationship has been established." We investigated dose-response curves in several cases.² There were five different doses from 0.015 ml. to 0.240 ml. given intravenously. Platelet count increases higher than those quoted by Dr. Penington were produced five to eight days after the plasma injection. Bone marrow megakaryocytes were also investigated. At least some pathologically active human plasma samples initiated a significant bone marrow and splenic megakaryocytosis, with a shift to the left in recipient mice already one day after the appropriate stimulus. The same pattern of changes followed the administration of "positive" mouse plasma. Several conditions of platelet physiopathology were, later, investigated and bone marrow changes, in general, were in accordance with expectations, except post-splenectomy thrombocytosis.⁵

It is surely unfair to disparage extensive previous work because some preliminary results with a seemingly more precise method diverged; especially as Penington's new method, used independently by others,⁶ appears to be a real advance.—I am, etc.,

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- 5 Krizsa, F., Gergely, G., and Rák, K., in press.
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Glaucoma

SIR,—I feel sure that practitioners who have read Mr. S. Romano's article on primary glaucoma (16 May, p. 407) will be grateful for his conciseness and clarity. He has, however, confined the article to glaucoma in the adult.

I think a description of primary glaucoma ought to remind the reader of the congenital form, which is not an uncommon condition. It may be classified apart from glaucoma associated with rarer diseases occurring in fetal or neonatal life, and which diseases exhibit either purely ocular defects or these together with systemic abnormalities.

Most cases of congenital glaucoma are sporadic but a small percentage show an autosomal recessive trait. Mechanical impairment of aqueous outflow, with a

resultant rise in intraocular pressure, produces early symptoms and their recognition is important. They appear before the physical signs and are an intolerance of bright light, blepharospasm, and epiphora. Later the signs appear of a hazy cornea in an enlarged eye which feels hard when palpated, becomes somewhat injected, and in which the optic disc may be seen to be cupped. The treatment is essentially surgical, supplemented where necessary by miotics, and the correction of refractive errors.

Since the prognosis depends on not only early treatment but also early recognition of the disease, one should therefore consider the possibility of congenital glaucoma when one is asked to see an infant who is persistently photophobic and irritable, and an ophthalmic opinion may be very welcome.—I am, etc.,

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Illustrated Lectures

SIR,—Having just returned from a medical conference at which the majority of the speakers used slides during their talks, I wonder if I might have the courtesy of your columns to draw attention to the uselessness of the bulk of these slides because of their illegibility.

Since many participants at such conferences travel far and at much—usually public—expense in order to attend, it would

seem desirable that as much as possible of what is addressed to them should be assimilable.

High quality slides require the specialist medical illustrator's skill. But if the following points are borne in mind, any speaker can produce useful slides.

Make as few points per slide as possible; more than five is probably too many. Use several slides instead.

Represent the information in diagrammatic form if at all feasible, rather than in words and numbers. Simple histograms and graphs are most effective, using thick lines. Omit everything that is not essential.

If using a typewriter, the area to be photographed should be no larger than 3.5 in. × 5 in. (appr. 9 cm. × 12.5 cm.)—assuming a pica typeface with 10 characters to the inch. An electric typewriter with carbon ribbon is most desirable.¹

Lettering on larger originals than the size mentioned should be clearly legible when the original is viewed from a distance equal to eight times its largest (that is diagonal) dimensions—for quarto paper, from over 4 yards (3.7 m.).

Positive—that is black on white—slides are on the whole more legible than negative.²—I am, etc.,

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Diazepam and Neuromuscular Transmission

SIR,—Drs. S. A. Feldman and B. E. Crawley have recently reported that diazepam potentiates the neuromuscular blockade produced by gallamine (9 May, p. 336). This is in contrast to the findings of Hunter¹ who investigated diazepam and tubocurarine.

In order to determine whether diazepam affects transmission at the neuromuscular junction we have investigated its activity upon the rat phrenic nerve-diaphragm

preparation in vitro. Diazepam in concentrations between 2 and 16 × 10⁻⁵M potentiated tache inhibition was compared with the original control contraction height prior to diazepam pretreatment. However if this percentage inhibition was expressed in terms of the contraction height after diazepam pretreatment a slight potentiation of neuromuscular blockade was seen (13%).

In order to determine whether diazepam increased contraction height pre- or post-

Neuromuscular blockade produced by tubocurarine before and after pretreatment with diazepam. (T=tubocurarine 10⁻⁴M; D=diazepam 4 × 10⁻⁵M.)

contractions elicited by supramaximal stimulation of the phrenic nerve. This effect was dependent upon concentration and, at the highest concentration examined, a 50% increase in contraction height was seen.

Neuromuscular blockade produced either by tubocurarine or by gallamine was not potentiated by diazepam when the percen-

synaptically diazepam was added to the tissue bath with a high concentration of tubocurarine, and the muscle was stimulated directly. A sustained increase in contraction height was observed as before.

It is concluded that diazepam does not potentiate the effects of competitive neuromuscular blocking agents in vitro in

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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- 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