BRITISH MEDICAL JOURNAL

LONDON SATURDAY MARCH 12 1960

STAPHYLOCOCCAL INFECTION: ANTITOXIC IMMUNITY*

BY

H. J. PARISH, M.D., F.R.C.P.Ed.

AND

D. A. CANNON, O.B.E., M.B., D.T.M.&H.

Wellcome Research Laboratories, Beckenham, Kent

This difficult topic may fittingly be introduced by a brief consideration of the role of antitoxic immunity in various infections other than staphylococcal. In diphtheria and tetanus the presence of antitoxin in sufficient quantity at the right time in the circulating blood is synonymous with immunity. In scarlet fever also—fortunately now a rare infection—antitoxin is of value in preventing the rash due to streptococcal toxin, and in causing it to disappear rapidly if it is already present at the time of administration. However, in other streptococcal diseases, in the era before effective chemotherapy was introduced, antitoxic serum was tried as soon as it was realized that the erythrogenic toxins of the haemolytic streptococci were for practical purposes identical (Okell and Parish, 1928; Okell, 1932); the small number of qualitative variations to which attention was subsequently directed has not invalidated the main thesis.

Results in treatment were conflicting at first because they rested on clinical impressions and not on any more certain means of scientific assessment. In retrospect, streptococcus antitoxin was certainly useful in a few cases of sepsis with a scarlatiniform rash, but in the ordinary run of puerperal infection, erysipelas, and general septicaemia the final verdict was that it was valueless—and that in spite of the laboratory evidence that antitoxic serum delayed the death of rabbits injected intravenously with large doses of living haemolytic streptococci from these conditions. As there was apparently no protection against virulent strains of streptococcus, the neutralization of the specific erythrogenic toxin was not a complete answer to the problem of streptococcal invasion.

Many of the toxic products of staphylococcal filtrates had been studied, mostly by German workers in the decade before 1905, but this early knowledge was largely neglected for nearly a quarter of a century. In 1928, a few years after the corresponding and in some respects similar investigation of haemolytic streptococcus toxins, research was resumed (Burnet, 1929, 1930, 1931): it had received a fresh impetus from the immunization disaster at Bundaberg, in Queensland, Australia, where 12 out of 21 children died from overwhelming staphylococcal toxæmia within 34 hours of receiving an injection of contaminated diphtheria prophylactic to which no preservative had been added.

*Based on a paper read by one of us (H.J.P.) at the Symposium on "The Staphylococcus" at the Postgraduate Medical School, Hammersmith, July, 1959.

A further incentive to research was derived from the analogy between staphylococcus alpha toxin and diphtheria, tetanus, and haemolytic streptococcus toxins, and also the ease with which this toxin and its corresponding antibody can be demonstrated and measured with accuracy. It was fitting to inquire how far the experimental findings in rabbits, guinea-pigs, and mice, in which the neutralization of toxin has a demonstrably beneficial effect, could be applied to the prophylaxis and treatment of staphylococcal disease. Notwithstanding the failure with a streptococcus antitoxin to prevent streptococcal invasion in man, there was considerable optimism that, even if all toxins and other factors in the attack of the staphylococcus were not combated directly, once the potentially dangerous alpha toxin was out of the way the body's defences would be enabled to deal with the others.

In this communication it is impossible to survey the considerable literature of toxin, toxoid, and antitoxin: this has been admirably carried out by Elek (1959). Our purpose is to indicate in the space at our disposal what has been achieved and also the gaps in our knowledge necessitating further basic work. As a background to this task, we propose to deal with some of the experimental findings and the views expressed in various published articles and personal correspondence over three decades.

The gravity of staphylococcal septicaemia (excluding the irregular bacteraemia of osteomyelitis, etc.) was much discussed in 1928 at the time of the Bundaberg disaster, which was the starting-point of Burnet's work on the alpha toxin and is therefore of special relevance to this review. The following data have been abstracted from the early literature:

<i>Staphylococcus Septicaemia</i>		
	No. of Cases	Mortality
Otten (1907)	55	80%
Soper (1912)	40	72%
Jochmann (1924)	23	95.6%
Bundaberg (Queensland) disaster in 1928	21	57%
Lowenstein (1931)	57	61.4%

Thus, true staphylococcal septicaemia had a case-fatality of the order of 60 to 95%.

Staphylococcus Alpha Toxin

The method developed for preparing staphylococcus toxin at the Wellcome Research Laboratories (Parish and Clark, 1932) was one of several variations of the

5175

procedure introduced by Burnet (1929, 1930), and may be summarized as follows:

The "Wood 46" strain is employed, as has been customary in most serological laboratories. Isolated in Australia from extensive infected burns, this is a consistently high producer of alpha toxin, analogous in its reputation to the "Park William 8" strain in the production of diphtheria toxin. The culture medium is 0.8% ("sloppy") agar under 25% CO₂. After 48 hours' incubation, modified Parker's broth is added: this contains 4% Witte peptone or Difco proteose peptone. The bottles are shaken to break up the agar. After a further period of incubation for four days under 25% CO₂, the cultures are harvested: the filtrate contains the alpha toxin.

Tasman *et al.* (1952) and Tasman and van der Slot (1953) have investigated and modified this method at the National Institute for Public Health, Utrecht. While confirming that CO₂ is indispensable, they obtained better results with a softer agar—0.5% instead of 0.8%. Their experiments suggested that the addition of Parker's broth in the second phase of the preparation was unnecessary. It was first replaced by 3% casamino acids, and later by normal saline, which was an equally effective extractive at lower cost and with a lower nitrogen content. This ensured greater purity of the product, apparently without any loss of stability, and facilitated further purification.

Many other types of media and procedure are reviewed by Elek (1959).

Staphylococcus Toxoid: Preparation and Purification

At the Wellcome Research Laboratories the alpha toxin is treated with 0.2% formaldehyde for 10 days at 37° C. Tasman and van der Slot (1953) found that the alpha toxin which they extracted from soft agar with saline detoxified a little more quickly than when extracted with broth or casamino acids. Toxoids prepared by either method are very stable.

Since the toxoids used originally were impure preparations and caused reactions, various attempts were made to improve them by purification. Holt (1936) salted out with ammonium sulphate and obtained an 80 to 90% yield. Burnet and Freeman (1932) tried precipitation with acetic acid, and Boivin and Izard (1937) precipitation with trichloroacetic acid. Wittler and Pillemer (1948) used fractional precipitation in methanol-water mixtures at accurately controlled temperature (below freezing-point), pH, and ionic strength. Tasman *et al.* (1952) employed ultrafiltration and rejected acid precipitation since it damaged the antigen. Tasman, in a personal communication in 1959, informed us that he has been using ultrafiltration and salting-out with ammonium sulphate for about two years. The toxoid is adsorbed on to aluminium sulphate (10 Lf and 3 mg. AlPO₄ per ml.). Much has been achieved by these modifications, because Tasman states that his ultrafiltered and adsorbed preparation produces *no unpleasant reactions*. Work on purified toxoid is in progress in other laboratories also, and more potent and milder preparations can now be investigated clinically than were available in the 1930s.

Antitoxic Immunity in Animal Experiments

Alpha toxin kills rabbits, guinea-pigs, and mice rapidly on intravenous injection; it produces necrotic

lesions on intradermal injection into rabbits and guinea-pigs; it has a haemolytic activity for rabbit red blood cells proportional to its toxicity; and it has a destructive action on rabbit leucocytes, but practically none on human leucocytes. It has to be differentiated from the true leucocidin (Panton and Valentine, 1932; Valentine, 1936), which is destructive for both human and rabbit leucocytes but without action on red cells.

Alpha antitoxin (horse, rabbit, or human) has protective properties which can be investigated by (a) a rabbit intravenous method, (b) a skin-neutralization method (guinea-pig), and (c) the titration of anti-haemolytic properties. The measurement of staphylococcus antibody by a mouse-protection test is being reinvestigated by Fisher (1959).

As the alpha antibody is present in the pseudoglobulin fraction of the serum, it has been found possible to concentrate horse antitoxin by the usual method of pepsin treatment.

The immunization of rabbits or horses with toxoid or toxin produces high titres of circulating antitoxin. When immunized rabbits are challenged by intradermal or subcutaneous injection of virulent culture, the resulting lesions show a minimum amount of necrosis, are less extensive, and heal more rapidly than those produced in controls. Immunized animals also outlive controls on intravenous challenge with a lethal intravenous dose, but sterilization of the tissues is not consistently obtained and metastatic abscesses ultimately develop in the viscera or joints. The immunity is antitoxic rather than antibacterial. We have already indicated the close analogy here between the value of staphylococcus antitoxin and streptococcus antitoxin in protection experiments in rabbits; streptococcus antitoxin also neutralizes the toxin in living broth cultures of toxigenic haemolytic streptococci, but while the lives of the "protected" animals are prolonged they almost all develop streptococcal arthritis, with pus in or around one or more joints (Parish and Okell, 1927): the serum is deficient in antibacterial properties. Thus, in both streptococcal and staphylococcal infections, humoral antibody is not the whole story, and something else, of which we have scarcely an inkling, is needed to dispose of the invader.

Downie (1937) failed to demonstrate any immunity in response to suspensions of *washed* heat-killed staphylococci: animals so injected reacted like the normal controls when challenged with toxin or living organisms; washed vaccines may therefore have lost important protective antigens in the washings. Perhaps when formalized and other "whole" vaccines give rise to some immunity in man, at least one factor may be alpha toxin or toxoid or both in their supernatant, which would stimulate the production of alpha antitoxin on injection. However, a rise in antitoxin titre does not ordinarily follow treatment of animals or patients with staphylococcus vaccines, and, in general, vaccines have been regarded as inferior to toxoids as immunizing agents (Kitching and Farrell, 1936).

Antitoxic Immunity in Man

The results obtained with staphylococcus toxoid and antitoxin in animal experiments have been paralleled to a limited extent in the prevention or treatment of human disease, but antitoxic immunity appears to be even less important in man than in the rabbit. As Elek (1959) points out, the alpha toxin is a leucocidin to the

rabbit, which may explain why this species may derive certain advantages from antitoxin which are denied to man.

In examining the problem the following question may be postulated. What is the relationship (if any) between circulating alpha antitoxin and clinical protection? How good are the results with toxoid and antitoxin in man, and could they be improved by the use of purer and more potent antigens, perhaps with some adjuvant? Relatively poor toxoids may have been used for immunization procedures in early investigations (Glenny and Stevens, 1935). A purified alpha toxoid would be less likely than crude toxoid to cause reactions in man, and also in horses undergoing immunization for the production of antitoxic serum; after purification, antigens would be more acceptable, could be used in larger doses if necessary, and should lead to better sera. What consideration should be given to antigens other than the alpha toxoid? Would better results be obtained by using different toxoids for immunization, or perhaps by mixing these antigens with some other constituents—possibly by combining toxoid and vaccine? Would the extra effort be justified on the evidence? We feel that we have only partial answers to these questions.

The wide range of values of *circulating antitoxin* within groups classified as "normal" humans and in groups of the various staphylococcal diseases is noteworthy. It is impossible to give a baseline or even a zone for normality and therefore to state what constitutes an abnormal finding. In general, patients with boils, blepharitis, acne, sycosis, and other superficial infections give values which are practically unaltered or only slightly above normal: the absence of any regular increase suggests that antitoxin is not an important factor in immunity. The values in cases of carbuncles, acute osteomyelitis, and purulent arthritis may be slightly or moderately raised, but here again the values do not correlate with the course of the disease. With chronic osteomyelitis, perinephritic abscess, pyaemia—what have been termed "deep-seated infections of bone and muscle"—the titres may be very high, though not always so. Low titres do not necessarily indicate inability of the patient to produce antibody, as many such patients respond well to toxoid.

Toxoid in Superficial Infections

In the early years of toxoid, much of the clinical investigation was carried out by clinical pathologists who were impressed by the considerable increase in anti-alpha-haemolysin titres—the haemolytic method of titration was employed—and by the concomitant recovery or remission from staphylococcal lesions, in the majority of subacute or chronic cutaneous infections. Some dermatologists who had long experience of the behaviour of various types of superficial lesion tended to be less enthusiastic, and thought that equally good results had often resulted from the non-specific measures in vogue at that time. Discrepancies appeared at once. Rises in circulating anti-haemolysin titres in response to toxoid were usual but by no means invariable; there was, in fact, no definite level of antitoxin which could be taken to demarcate a successful result from failure. It was difficult to understand why some patients with intractable furunculosis might have "normal" values of circulating staphylococcus antitoxin and yet have a history of remissions, the mechanism of which was obscure from the immuno-

logical standpoint. Clinicians, whose impressions were unfavourable to toxoid, were hesitant about publication, and their adverse comments seldom appeared in the literature. The controversy about the role of antitoxic immunity was still unsettled when chemotherapy made the topic unimportant but of rather academic interest.

One of us (H. J. P.) formed the impression that the toxoid used in the 1930s was often helpful, perhaps in 70% of cases. Some of the reports he received were impressive, and in a limited personal experience his successes outnumbered the failures: unfortunately, attempts to arrange controlled trials were abortive. The case of one woman, herself a well-known research worker in this field comes to mind. She had a history of recurrent carbuncles when she received a long course of toxoid with apparent success. Disappointment ensued when a further lesion developed; but fortunately, to her astonishment, a single small injection of toxoid cured it overnight. On two or three subsequent occasions, at intervals of several months, abscesses or carbuncles were kept at bay by these reinforcing doses given early in attacks. This impressive experience has not been unique, and it has been stated by protagonists of toxoid that recurrences after a course are usually milder and tend to respond rapidly to further injections.

Over a period of 10 years Ramon *et al.* (1946) recorded failure in only about 7% of approximately 3,000 cases of cutaneous infections treated with toxoid.

Belatedly, and as recently as 1951, Macdonald and Taylor were able to arrange a controlled clinical trial of toxoid on young R.A.F. personnel with pustular acne. Of 34 cases treated with toxoid, 21 (62%) showed improvement, whereas in the control group of 32 cases treated with broth, only 5 (16%) improved.

Toxoid in Deep-seated Infections

Since antitoxic immunity is, at best, only one aspect of defence against staphylococci, it is hardly surprising that the results obtained with toxoid in deep-seated infections, such as chronic osteomyelitis, have been uniformly disappointing (Blair, 1937). As already mentioned, the values of circulating alpha antitoxin in many cases of osteomyelitis are already unusually high, and further variations after the injection of toxoid do not correlate with the course of the disease.

Antitoxin in Treatment

The value of antitoxin therapy was subjected to the same controversy as toxoid: there is a considerable literature (Elek, 1959). Our views coincide with those of Blair (1958), as follows: "Staphylococcal antitoxin has a definite but restricted role, which is properly confined to fulminating systemic infections, especially in children, with evidence of severe toxæmia (Kleiger and Blair, 1943). When administered intravenously early and in adequate doses, antitoxin may carry the patient over the acute period of emergency. Upon survival of the acute phase of the infection, the clinical picture becomes chiefly that of the effects of metastases to various tissues or organs. Antitoxin therapy in this phase is of little value, nor can it be expected to influence an infection due to a non-toxigenic strain."

The difficulties and pitfalls inherent in any attempt to influence staphylococcal infections by specific measures are illustrated once again by the above quotation. What are some of the gaps in our knowledge left by previous neglect, and what research projects might be undertaken to fill them?

Criticism and Suggestions

In the preparation of antigens the selection of polyvalent strains might be rewarding. Hitherto too much attention may have been given to the "Wood 46" strain, and therefore to the alpha toxoid or haemolysin it produces. It is not known whether better results would be obtained if, in addition to the alpha toxoid, beta and possibly gamma and delta toxoids were included, and also strains of staphylococci—or preferably one autogenous strain—in other words, if, together with a more comprehensive antitoxic immunity, some antibacterial immunity were sought as well. The antigenicity of the specific coagulase should be considered in the selection of strains. More attention, too, might be paid to the anti-leucocidin for human leucocytes, as suggested by Pantón and Valentine (1932) and Valentine (1936). The enterotoxin which is associated with staphylococcal food-poisoning is different from these other toxins and does not appear to be antigenic.

Dolman (personal communication, 1959) considers that "strains of staphylococci currently prevalent in hospital 'reservoirs' . . . might elaborate labile or elusive antigens (leucocidins, for instance) which could be very important. In any event such antigens are unlikely to be present in the monovalent alpha toxoid now being manufactured, as the 'Wood' strain has been through many subcultures since its first isolation 30 or so years ago. In the early days, the Connaught Laboratories' toxoids were made from the pooled filtrates of some six to ten strains, which included always one or two freshly isolated ones. The results obtained from these polyvalent antigens were certainly better than those given by the monovalent product. Of course the earlier toxoid was much harder to make and standardize; and introduction of the standard unit of alpha-antitoxin encouraged the use of material of single-strain origin. For some time I have been trying out, under Connaught Laboratories' auspices, a bivalent toxoid, in which both alpha and beta toxoids are present in known proportions. The clinical results have been encouraging in some cases of recurrent infection which proved intractable to alpha toxoid and antibiotics. But I feel that even the bivalent toxoid does not measure up to the 1932-5 results in Toronto—either because nowadays the flora is more versatile, or because the antigens are less comprehensive."

Mercier (personal communication, 1959) also favours the use of a multiplicity of antigens. He makes the interesting suggestion that antibiotic therapy should always be accompanied by the most complete immunization possible—namely, heat-killed staphylococci of the Cowan types most frequently found in the area, suspended in purified toxoid. As the immunity is transitory, frequent boosting doses are necessary.

Murray (personal communication, 1959) is still an enthusiastic user of toxoid, either alone or as a constituent of vaccines ("vaccoids").

It follows from the brief review in earlier sections of this article that the medium used for toxin production and the rationale of growth could be improved by the type of intensive study which has proved so fruitful in the production of, say, diphtheria toxin or pertussis vaccine. Further, a purified toxoid would reduce the incidence of untoward reactions, permit larger dosage and fewer injections, and induce better antibody

response. Considerable work on adjuvants is also indicated.

Some of the poor results with toxoid have been ascribed to weak or otherwise unsatisfactory preparations. It has been suggested that, by analogy, the control of diphtheria and whooping-cough would have been failures if only weak antigens had been available. Alternative explanations have also been advanced—for example, Branch (1938) felt that once a focus of infection had become established toxoid gave no protection. Elek (1959) makes the acid comment that "the old clinical maxim has been applied to toxoid treatment: 'Useful in carefully selected cases.' It should be added, however, that the selection has usually had to be made in retrospect."

Attention has been drawn by Stuart-Harris (1959) and others to the importance of the staphylococcus in cases of pneumonia complicating the epidemic of Asian influenza of 1958. This organism was found in 62% of the British and 67% of the Dutch necropsies. Resistance to penicillin was common. There was no evidence of bacteraemia. Often death appeared to be due to the toxic effects of the infection. Though antibiotics are of course useless for the bacterial toxæmia of these complicated cases, Stuart-Harris concluded that staphylococcus antitoxin had not "really justified its claim for inclusion in the therapeutic regime."

Dolman (1956) has mentioned some errors and difficulties in the administration of antitoxin and in assessing the results. While antitoxin had been valuable in severe and even desperate acute cases in Montreal (Dolman, 1934), some doctors appeared to expect small doses to cure patients riddled with abscesses, perhaps in sites inaccessible to circulating antitoxin. In other cases large doses were given in the late stages of the infection when damage was already irreparable. More recently, Dolman (personal communication, 1959) has not lost his early conviction that "toxins of one sort or another play a big part in the pathogenic activities of staphylococci. . . . If antitoxin were used earlier in larger intravenous doses, it would be very useful, particularly in the fulminating bronchopneumonia and enterocolitis cases which are so baffling to cope with otherwise." Dolman deplores once again that, though antitoxin is used in many hospitals in Canada, it is given only on rare occasions: "presumably the evidence of 'toxæmia' is overwhelming in fulminating cases, and when a patient is at death's door there is willingness to try out anything that sounds 'specific.'"

Murray (1935; personal communication, 1959) agrees with Dolman that antitoxin, given quickly enough with or without an antibiotic, is often valuable in acute infections.

A rare staphylococcal "scarlet fever" has been described: possibly the rash is due to a toxin which might be controlled by antitoxic serum. Essential information is lacking (Negro *et al.*, 1956).

Gamma-globulin.—Ede *et al.* (1959) used gamma-globulin in critically ill cases of staphylococcal pneumonia, but were unable to judge its *real* efficacy because other agents were being given at the same time.

Allergy.—While repeated injections of living cultures of staphylococcus will immunize rabbits, they will at the same time cause hypersensitivity to small doses which have no effect on normal animals (Pantón and Valentine, 1929); thus immunity may coexist with hypersensitivity in staphylococcal as in other infective conditions.

Though several papers have been published on the part played by allergy in staphylococcal infections (Elek, 1959), nothing of practical value has so far emerged from experiments involving allergy. This line of investigation is unpromising.

Conclusion

Antitoxic immunity is only one factor in the control of infection generally. There is a close parallel between staphylococcus antitoxin and haemolytic streptococcus (scarlet fever) antitoxin in that both protect rabbits against intravenous lethal doses of culture without disposing of all the invaders: temporary recovery from the inoculation is followed by abscess formation (metastases) in various sites.

In man, also, staphylococcal and streptococcal infections may persist in spite of procedures designed to raise the concentration of serum antibodies to the highest possible level. Nevertheless, there is evidence, which appears adequate, that staphylococcus antitoxin will prevent some fatalities from staphylococcal infection in human subjects, and that the administration of alpha toxoid may be useful in many cases of superficial infection.

Suggestions have been made in this paper for "improving" antigens and antibodies in various ways. But humoral immunity is not the full story: there is an elusive "something else," and the study of staphylococcal infection—and indeed of infection generally—"will have taken a great step forward when the nature of this other factor is brought to light" (*Brit. med. J.*, 1937).

REFERENCES

- Blair, J. E. (1937). *Proceedings of the Second International Congress of Microbiology, London, 1936*, p. 518.
 — (1958). In *Bacterial and Mycotic Infections of Man*, edited by R. J. Dubos, 3rd ed., p. 325. Pitman, London.
 Boivin, A., and Izard, Y. (1937). *C.R. Soc. Biol. (Paris)*, **124**, 25.
 Branch, A. (1938). *Canad. med. Ass. J.*, **39**, 391.
Brit. med. J., 1937, **2**, 860.
 Burnet, F. M. (1929). *J. Path. Bact.*, **32**, 717.
 — (1930). *Ibid.*, **33**, 1.
 — (1931). *Ibid.*, **34**, 471.
 — and Freeman, M. (1932). *Ibid.*, **35**, 477.
 Dolman, C. E. (1934). *Canad. med. Ass. J.*, **30**, 601; **31**, 1, 130.
 — (1956). *Canad. J. Microbiol.*, **2**, 189.
 Downie, A. W. (1937). *J. Path. Bact.*, **44**, 573.
 Ede, S., Davis, G. M., and Holmes, F. H. (1959). *J. Amer. med. Ass.*, **170**, 638.
 Elek, S. D. (1959). *Staphylococcus Pyogenes and its Relation to Disease*. Livingstone, Edinburgh and London.
 Fisher, M. W. (1959). *Nature (Lond.)*, **183**, 1692.
 Glenn, A. T., and Stevens, M. F. (1935). *J. Path. Bact.*, **40**, 201.
 Holt, L. B. (1936). *Brit. J. exp. Path.*, **17**, 318.
 Jochmann, G. (1924). *Lehrbuch der Infektionskrankheiten*, 2nd ed. Springer, Berlin.
 Kitching, J. S., and Farrell, L. N. (1936). *Amer. J. Hyg.*, **24**, 268.
 Kleiger, B., and Blair, J. E. (1943). *Arch. Surg. (Chicago)*, **46**, 548.
 Lowenstein, P. S. (1931). *Amer. J. med. Sci.*, **181**, 196.
 Macdonald, T. C., and Taylor, F. (1951). *Lancet*, **2**, 558.
 Murray, D. S. (1935). *Ibid.*, **1**, 303.
 Negro, R. C., Gentile-Ramos, I., and Galiana, J. (1956). *An. Fac. Med. Montevideo*, **41**, 263.
 Okell, C. C. (1932). *Lancet*, **1**, 761, 815, 867.
 — and Parish, H. J. (1928). *Ibid.*, **1**, 748.
 Otten, M. (1907). *Dtsch. Arch. klin. Med.*, **90**, 461.
 Pantón, P. N., and Valentine, F. C. O. (1929). *Brit. J. exp. Path.*, **10**, 257.
 — (1932). *Lancet*, **1**, 506.
 Parish, H. J., and Clark, W. H. M. (1932). *J. Path. Bact.*, **35**, 251.
 — and Okell, C. C. (1927). *Ibid.*, **30**, 521.
 Ramon, G., Richou, R., Mercier, P., and Holstein, G. (1946). *Rev. Immunol. (Paris)*, **10**, 71.
 Soper, W. B. (1912). *Proc. N.Y. path. Soc.*, **12**, 223.
 Stuart-Harris, C. H. (1959). *Brit. med. J.*, **1**, 1606.
 Tasman, A., Brandwijk, A. C., Marseille, A., van Ramshorst, J. D., and van der Slot, J. (1952). *Antonie v. Leeuwenhoek*, **18**, 336.
 — and van der Slot, J. (1953). *Ibid.*, **19**, 256.
 Valentine, F. C. O. (1936). *Lancet*, **1**, 526.
 Wittler, R. G., and Pillemer, L. (1948). *J. biol. Chem.*, **174**, 23.

OBSERVATIONS ON 100 CASES OF LEUKAEMIA IN CHILDHOOD

BY

REGINALD LIGHTWOOD, M.D., F.R.C.P.
Consultant Physician

HERBERT BARRIE,* M.D., M.R.C.P.
Assistant Medical Registrar

AND

NEVILLE BUTLER,† M.D., M.R.C.P.
Medical Registrar and Pathologist

The Hospital for Sick Children, Great Ormond Street, London

[WITH SPECIAL PLATE]

Deaths attributed to leukaemia at all ages in England and Wales have increased two and a half times in the last two decades (Registrar-General's Statistical Review, 1938–57). In childhood there is now a peak incidence between the ages of 2 and 4 years (Hewitt, 1955). Acute leukaemia accounts for nearly 25% of cases of malignant disease admitted to the Hospital for Sick Children, Great Ormond Street. In this paper a survey of clinical and radiological findings is presented and an analysis made of the form and duration of remissions induced by antileukaemic agents. Steroids are prominent in treatment, and the place of antimetabolites has been firmly established since the original studies of Farber *et al.* (1948) on folic-acid antagonists and of Burchenal *et al.* (1953) on 6-mercaptopurine. Remission therapy can lengthen life and can change the clinical course of the disease. It can also affect pathology; references to infiltration of the kidneys (Pierce, 1957) and intracranial structures (Sullivan, 1957) are made in this paper.

Material

This report is based on 100 cases admitted to the Hospital for Sick Children between September, 1951, and March, 1957. The diagnosis was confirmed by marrow examination in every case. Twenty other children attending with leukaemia during the same period are omitted because 17 of them were treated elsewhere after confirmation of the diagnosis and three were suffering from lymphosarcoma with leukaemic termination.

TABLE I.—*Age and Sex Distribution in 100 Cases of Leukaemia in Childhood*

Age on Admission (Years)	Males	Females	Total
0–1	1	3	4
1–2	3	4	7
2–4	15	10	25
4–6	10	12	22
6–8	11	12	23
8–10	7	6	13
Over 10*	4	2	6
Total	51	49	100

* Twelve years is maximum age for admission.

The age and sex distribution are shown in Table I. Sex incidence was equal. Aetiological factors were not investigated, as those children who died between 1953 and 1955 had already been included in a study on the antecedents of childhood leukaemia (Stewart *et al.*,

*At present Paediatric Senior Registrar, St. Thomas's Hospital, London.

†At present Consultant Paediatrician, Oxford and S.W. Metropolitan Boards.