PAPERS AND ORIGINALS

Thrombolytic Therapy in Haemolytic—Uraemic Syndrome

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Summary

The treatment of five children with the haemolytic-uraemic syndrome using streptokinase is described to illustrate the difficulties and limitations of thrombolytic therapy in this disease. This experience is germane to the design of multi-centre clinical trials.

A multivariate analysis relating clinical outcome to the data obtained at the time of admission was also carried out for 31 children with the disease treated in four centres. The results suggest that this technique may help to identify those patients likely to have a fatal outcome. An expanded form of this type of analysis should be incorporated in future clinical trials.

Introduction

Since the first description of the haemolytic-uraemic syndrome by Gasser *et al.* (1955) the reported mortality has fallen from 67% (Shinton *et al.*, 1964) to levels of 4.5% (Tune *et al.*, 1973) and 6.3% (Gianantonio, *et al.*, 1973). This fall in mortality has coincided with better management of the acute renal failure and, in particular, with the introduction of peritoneal dialysis. It has also been attributed in some cases to the use of heparin (Moncrieff and Glasgow, 1970), but there is no convincing evidence from recent reports (Kaplan *et al.*, 1971; Vitacco *et al.*, 1973) that heparin is of additional value to good supportive therapy in most cases.

Thrombolytic therapy with streptokinase has recently been advocated as a means of lysing intrarenal fibrin and thereby improving renal perfusion (Monnens *et al.*, 1972; Bergstein *et al.*, 1972; Winterborn *et al.*, 1972). Experience has been

limited to a few cases only except for one unpublished group of 58 patients treated consecutively, in which the acute phase mortality was 12%. Only one of 34 patients followed for more than two years subsequently showed long-term sequelae of hypertension and impaired renal function, however (Monnens, 1974). This contrasts with the high incidence (52%) of residual abnormality in 124 Argentinian patients followed for more than five years (Gianantonio *et al.*, 1973).

There are, however, considerable difficulties in the use of streptokinase in young children undergoing peritoneal dialysis and the indications for this drug and its clinical value need to be evaluated by clinical trial (Winterborn *et al.*, 1972). In this paper we describe our experience with five children with the haemolytic-uraemic syndrome, who illustrate the difficulties in management and the limitations of streptokinase therapy. These difficulties and limitations are relevant to the design of controlled clinical trials in this disease. We also attempted to define criteria of prognostic significance in 31 children with the syndrome to identify severely affected patients who might benefit most from thrombolytic therapy.

Patients and Methods

Clinical and laboratory details of the patients studied are given in table I. A diagnosis of haemolytic-uraemic syndrome was made on the basis of anaemia, red cell fragmentation, thrombocytopenia, raised serum fibrin degradation products (F.D.P.), and acute renal failure; the preceding illness included diarrhoea and vomiting in all cases. The diagnosis was confirmed in four children by renal biopsy or at

TABLE I—Clinical and Laboratory Data at Time of Admission in Five Patients with Haemolytic-uraemic Syndrome

	Normal Range	Case No.				
		1	2	3	4	5
Age (months)		49	119	27	23	14
Duration of illness (days)		7	3	3	7	14
Hb (g/100 ml)	10.5-14.0	3.6	5.3	8.4	6.4	4.6
Platelets ($\times 10^9/l.$)	140-500	54	30	35	266	40
Serum F.D.P. ($\mu g/ml$)	0-10	112	56	160	30	30
Thrombin time (sec)	10-12	15	20	14	12	20
Fragmented red cells (%)	0-5	Many	Many	10	30	18
Serum creatinine (mg/100 ml)	0.3-1.3	10.1	5.6	7.6	2.6	6.0
Oliguria or anuria		+	—	+	—	+

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necropsy according to the morphological characteristics described by Habib *et al.* (1969). None of the patients had septicaemia or hyperosmolar dehydration. All patients received full supportive therapy including peritoneal dialysis. The decision to use streptokinase was based on clinical assessment of the severity of the disease.

THROMBOLYTIC THERAPY

Since none of the children showed resistance to streptokinase (Streptase) an initial dose of 20 U/ml blood volume (estimated as 80 ml/kg body weight) was given intravenously over 30 minutes. The same dose was then given hourly, using a Watson-Marlow constant infusion pump, for 12 hours. Thereafter the dose was adjusted to maintain a thrombin time of 2-4 times the laboratory control value for pooled normal plasma; the thrombin concentration used was 10 N.I.H. (National Institutes of Health) units per ml and the solution contained 0.035 mol calcium chloride per litre. The hourly dose of streptokinase was decreased to 25-30% of the initial dose in four of the five patients to maintain a thrombin time, measured every 12-24 hours, of greater than twice the control value. Streptokinase was given for 72 hours to three patients and for 120 hours to two.

Heparin therapy was started 4-12 hours after the streptokinase infusion had been stopped. It was given by continuous infusion at an initial dose of 25 U/kg/hr and adjusted to maintain the thrombin time (measured daily or twice daily) at 2-4 times the laboratory control value. Two patients remained thrombocytopenic and oliguric at the end of nine and eleven days of heparin therapy and were given oral warfarin plus dipyridamole thereafter.

COAGULATION STUDIES

Platelet counts were determined by phase-contrast microscopy (Dacie and Lewis, 1968), plasminogen by the caseinolytic method of Alkjaersig *et al.* (1959), fibrinogen by titre (Sharp and Eggleton, 1963) or by the method of Ratnoff and Menzie (1951) as modified by Alkjaersig (1960), factor V by a one-stage method after adsorption with aluminium hydroxide (Hardisty and Ingram, 1965), and F.D.P. by the method of Merskey *et al.* (1966) as modified by Das *et al.* (1967).

PROGNOSTIC INDEX

In order to study correlations between the clinical data on admission and the outcome of the illness a number of paediatric nephrologists were asked to supply details of children with the haemolytic-uraemic syndrome who had been under their care. Details of 41 children were obtained, including 18 of our own, but 10 had to be excluded from analysis because of insufficient information. A multivariate analysis was performed on the admission data for the remaining 31 children using the following twelve variates: age; sex; history of gastrointestinal symptoms in the preceding illness; blood level of haemoglobin, platelets, and urea; and the presence or absence of purpura, hepatomegaly, hypertension, convulsions, oliguria, and anuria. There were insufficient data to analyse serum F.D.P., serum creatinine, serum transaminases, percentage fragmented red cells, or urinary urea.

Results

STREPTOKINASE THERAPY

Details of treatment and outcome in the five children

treated with streptokinase are given in table II. Streptokinase was started within a few hours of admission and within seven days of the onset of gastrointestinal symptoms in two patients (cases 1 and 3). The patient in case 1 never became hypertensive and showed substantial recovery of renal function within one month. Renal biopsy on day 34 showed fairly diffuse glomerular capillary wall thickening but no evidence of active intravascular thrombosis, and only four out of 43 glomeruli appeared irreversibly damaged. After two years' follow up he had normal blood pressure, urine, and renal function.

TABLE II—Details of Treatment and Outcome in Five Patients treated with Streptokinase

Case No.	Therapy	Days Given*	Outcome
1	S.K.	7- 12	At 21 months: blood pressure 80/50 mm Hg, urine protein free, G.F.R. 107 ml/min/1.73 m ²
	H.	12- 17	
	P.D.	13- 16	
2	H.	21- 30	Died from hypertensive heart failure and renal insufficiency at 17 weeks
		36- 44	
		31- 36	
	S.K.	43-116	
		9- 14	
	W.&D.	34- 42	
3	P.D.	45- 49	Died with intra-abdominal haemorrhage on day 9
		114	
4	S.K.	5- 8	Died from hypertensive heart failure and renal insufficiency at 11 weeks
	H.	8- 9	
	P.D.	5- 9	
5	S.K.	13- 16	At 14 weeks: blood pressure 110/60 mm Hg, urine protein free, G.F.R. 125 ml/min/1.73 m ²
	H.	16- 26	
	W.&D.	24- 66	
	P.D.	31- 33	
		42- 46	
	S.K.	15- 18	
	H.	18- 29	
	P.D.	31- 41	
		16- 22	

S.K. = Streptokinase. H. = Heparin. P.D. = Peritoneal dialysis. W.&D. = Warfarin plus dipyridamole. G.F.R. = Glomerular filtration rate.
*As measured from the onset of gastrointestinal symptoms.

The patient in case 3 died as a result of cardiac arrest complicating intraperitoneal bleeding 29 hours after the onset of heparin therapy. A peritoneal dialysis catheter blocked with blood clot had been removed but not replaced because of clinical and biochemical improvement 16 hours before death. Seven hours before death routine laboratory tests gave a thrombin time of 20 sec (control 14 sec), a platelet count of $230 \times 10^9/l.$, serum F.D.P. of $< 2 \mu g/ml$, and plasminogen of 2.0 casein units. Despite these satisfactory results there had been inadequate local haemostasis at the site of insertion of the catheter, presumably because of previous clot lysis. At necropsy a large haematoma was found in the anterior abdominal wall in addition to heavily blood-stained fluid in the peritoneal cavity. She remained normotensive and showed a reduction in blood urea level from 324 mg to 176 mg/100 ml during her admission. Post-mortem kidney sections showed moderately severe glomerular involvement, but without irreversible sclerosis, and marked interstitial oedema. Immunofluorescence studies, however, showed massive intracapillary fibrin deposition.

One patient (case 5) was transferred from another hospital and did not begin streptokinase until 15 days after the onset of gastrointestinal symptoms; he had been anuric since day 12. The platelet count, which was $40 \times 10^9/l.$ on admission, had risen to $394 \times 10^9/l.$ by day 29, when heparin was stopped after continuous infusion for 11 days. The count fell to $83 \times 10^9/l.$ over the next four days (table III) and heparin was therefore restarted for a further 10 days. By then the platelet count had risen to $410 \times 10^9/l.$ and it subsequently remained normal. A renal biopsy specimen obtained six weeks after onset showed evidence of intracapillary thrombosis with diffuse capillary wall thickening in only nine of 43 glomeruli. He remained normotensive throughout and at 14 weeks after onset had protein-free urine and normal renal function.

TABLE III—Coagulation Results in Two Patients who developed Thrombocytopenia at Relatively Late Stage of Illness

	Normal range	Case 4		Case 5	
Day of test*		10	13	27	31
Platelets ($\times 10^9/l.$)	140-500	267	90	394	83
Plasminogen (casein units/ml)	1.3-3.7	2.55	2.75	1.6	2.0
Fibrinogen { titre	1/160-1/320	1/80	1/160		
g/l.	1.5-3.8			2.5	2.85
Factor V (%)	50-200			60	80
Serum F.D.P. ($\mu g/ml$)	0-10	80	40	20	5

*As measured from the onset of gastrointestinal symptoms.

Two patients (cases 2 and 4), who both had high-output renal failure on admission, were not given streptokinase until four and two weeks respectively after the onset of gastrointestinal symptoms. During the four-week delay the patient in case 2 showed a rise in platelet count to normal on two occasions when heparin was given. The platelet count subsequently fell again despite continuation of heparin on the second occasion. A transient response was observed during streptokinase therapy with a rise in platelet count to normal and a slow rise in creatinine clearance from 1 to 20 ml/min/1.73 m² during the succeeding month. The patient in case 4 showed a progressive fall in platelet count from 267 to 90 $\times 10^9/l.$ over the three days before treatment with streptokinase (table III), but there was no improvement during therapy. Renal biopsy on day 32 showed diffuse glomerular involvement though immunofluorescence studies showed only slight fibrin deposition within the capillaries and afferent arterioles. At necropsy both kidneys showed extensive interstitial fibrosis, while the left kidney in addition showed severe acute pyelonephritis. Both children (cases 2 and 4) developed uncontrollable hypertension, heart failure, and renal insufficiency after 17 and 11 weeks respectively.

During treatment with streptokinase our patients tended to develop haematomas at the site of previous venepuncture and also oozed from cut-down sites. The application of a swab soaked in epsilon aminocaproic acid effectively controlled this local oozing. In cases 2 and 3 there was persistent blood staining of the peritoneal dialysate during streptokinase therapy.

STATISTICAL ANALYSIS OF ADMISSION DATA

In an attempt to detect inherent patterns in relation to clinical outcome the data were first subjected to a cluster analysis (Gower and Ross, 1969), which produced no clear structure, and then to a principal co-ordinates analysis (Gower, 1966; Ashton and Flinn, 1969). The latter method is a statistical technique which maximizes and displays in the best possible way any difference between patient groups.

The patients were divided into two groups according to outcome (see fig.). Group 1 comprised 13 boys and 12 girls who either recovered or died from non-renal complications (electrolyte imbalance, haemorrhage). Group 2 consisted of two boys and four girls who died from progressive renal failure. The results for individual patients were then added to the plots of the co-ordinates of the patient groups (Gower, 1968).

The results show considerable separation between the boys, and to a lesser extent the girls, in group 2 and the children of both sexes in group 1. The clinical variates which contributed most to the separation of the groups and were associated with a poor prognosis were: the presence of anuria, hepatomegaly, and purpura and the absence of preceding gastrointestinal symptoms. The limited laboratory data analysed contributed little to the separation of the groups. Group 1 patients were aged 1-117 months (mean 44 months) and group 2 patients 23-123 months (mean 78 months), but patient age was not a useful discriminant. In

contrast to the usual predominance of children aged less than 12 months (Lieberman, 1972) only 32% of the 31 children in our analysis fell within this age group; this may, however, have reflected the patient selection inherent in referral to specialist centres.

The effect of treatment was not taken into account in the analysis. In group 1 15 patients (60%) needed dialysis, and heparin was given to 16 (64%), streptokinase to one, urokinase via the renal arteries to one, and corticosteroids to one. In group 2 all six patients eventually needed dialysis, and four were given heparin, four steroids, and one streptokinase. The separation of group 2 from group 1 on the basis of the admission data was very clear, however, and this suggests that treatment did not have a major effect in influencing the outcome.

Principal co-ordinates analysis based on 12 variates obtained at time of admission for 31 children with haemolytic-uraemic syndrome. Each point represents one patient and the centroid of each patient group is shown (+). Two axes (co-ordinates) shown are those which showed greatest separation between groups, and they are scaled in standard deviation units which correct for unequal variance. Figure shows clear separation of group 2 children from children in group 1. ■ = Group 1 boys. x = Group 1 girls. □ = Group 2 boys. ○ = Group 2 girls.

Discussion

Children with the haemolytic-uraemic syndrome show very great differences in duration of antecedent illness, degree of oliguria or anuria, and speed of referral to specialist centres with facilities for dialysis. There are also substantial differences in the nature of the disease as described in different countries (Tune *et al.*, 1973), particularly when relatively large clusters of patients are found. This variation in clinical severity together with the recent decrease in acute phase mortality indicate the need for randomized clinical trials to assess the value of anticoagulant or thrombolytic therapy.

Clinical studies using heparin have not shown any convincing benefit over good supportive therapy alone though certain patients with continuing intravascular fibrin deposition on admission may benefit from its use. Laboratory studies to detect continuing intravascular coagulation have been conflicting but have usually shown normal or high coagulation factor levels despite persistent thrombocytopenia (Gilchrist *et al.*, 1969; Sanchez Avalos *et al.*, 1970; Katz *et al.*, 1971). Isotope labelling of platelets and fibrinogen has also failed to show continuing intravascular consumption, and the results of organ scanning have suggested that the persistence of thrombocytopenia may be due to splenic sequestration rather than renal deposition of damaged platelets (Metz, 1972; Katz *et al.*, 1973). A few cases of the syndrome studied early in the disease have, however, shown low levels of coagulation factors, suggesting continuing consumption, with a subsequent rebound to high levels (Willoughby *et al.*, 1972). It would therefore seem of value to carry out serial daily co-

agulation studies in patients with the haemolytic-uraemic syndrome receiving supportive therapy alone to detect any sub-population of patients with continuing intravascular coagulation who might benefit from anticoagulant therapy.

Thrombolytic therapy is potentially of more value than heparin since the amount of fibrin already deposited in the kidney at the time of admission is likely to determine the extent of subsequent cortical ischaemia. It is, however, difficult to determine the value of thrombolytic therapy in a disease with a decreasing mortality (Prentice and McNicol, 1973; Sherry, 1973), and evaluation in the syndrome must therefore depend on criteria other than acute-phase mortality—for example, follow up renal biopsy appearances and the subsequent incidence of chronic renal insufficiency and hypertension.

Our own patients illustrate the spectrum which extends from clinical recovery after streptokinase therapy to death from renal failure within four months. The two patients who progressed to renal failure were not given streptokinase until two and four weeks after the onset of the illness and this contrasts with the absence of long-term sequelae in the patients treated by Monnens (1974), who received streptokinase within a few hours of admission. While some major arterial occlusions may be lysed by streptokinase after a delay of several weeks (Poliwoda *et al.*, 1969) it is unknown whether long-standing intrarenal microthrombi can similarly be removed.

The renal morphological changes are difficult to interpret; the less extensive glomerular involvement observed in cases 1 and 5 is consistent with their clinical recovery but does not indicate whether this was due to or in spite of streptokinase therapy. The necropsy appearances in case 3 were also compatible with recovery of normal renal function though the massive amount of intravascular fibrin despite thrombolytic therapy was a disturbing observation. Ideally renal biopsy should be performed before treatment, but this is impracticable because of the thrombocytopenia, and it would preclude the use of streptokinase for at least seven days.

Many paediatricians may be reluctant to use streptokinase in presumed good-risk cases. Ideally a randomized trial which includes thrombolytic therapy should initially be confined to poor-risk patients, but attempts to make an accurate assessment of prognosis at the time of admission have so far met with little success (Lieberman, 1972). Kaplan *et al.* (1971) classified the disease into mild and severe grades but by their criteria our two patients who died from renal failure despite streptokinase therapy would have been classified as mild and the two patients who recovered as severe.

A multivariate analysis of our own prognostic criteria does, however, suggest that patients with a poor prognosis may be identifiable by this technique at the time of admission to hospital. The criteria available for this retrospective study were of necessity limited, and the prognostic value of this type of analysis could almost certainly be improved with additional laboratory data, including coagulation tests, and by increasing the number of patients on which the analysis is based.

Therapeutic trials in the haemolytic-uraemic syndrome are likely to be limited to a two-arm randomization owing to the small number of patients available for study even by a group of centres. There are strong indications that initial trials should include patients receiving only supportive therapy, including peritoneal dialysis. These patients would provide a base line for short-term mortality and long-term morbidity in the participating centres, which would facilitate the evaluation of thrombolytic therapy. Serial coagulation studies could also be performed on the patients receiving supportive therapy to determine the frequency of continuing intravascular coagulation at the time of admission. An effective prognostic index might simultaneously be evolved and the effect of thrombolytic/anticoagulant therapy on this index

would provide a further criterion for evaluating these agents.

There are, however, considerable practical difficulties in using streptokinase in these patients. The potential hazard of intraperitoneal bleeding during simultaneous peritoneal dialysis is a major one and the indications and timing of dialysis need to be clearly defined. It is clear that a patent peritoneal catheter must be left in situ while there is any risk of haemorrhage, even when laboratory control seems satisfactory.

If there is a continuing stimulus to intravascular coagulation, and therefore rethrombosis, in these patients complete plasminogen depletion must be avoided, and the control of streptokinase dosage is therefore critical. Three of our patients developed thrombocytopenia at different stages of treatment: before thrombolytic or anticoagulant therapy was given (case 4), after heparin alone (case 2), and after streptokinase followed by heparin (case 5). Serial coagulation studies in cases 4 and 5 at the time of development of thrombocytopenia (table III) did not show a parallel fall in plasminogen, fibrinogen, or factor V concentration or a rise in serum F.D.P. These results suggest that the thrombocytopenia was either an early and solitary indication of renewed fibrin deposition (which seems unlikely) or was due to adsorption on to pre-existing fibrin, splenic sequestration (Metz, 1972; Katz *et al.*, 1973), or an immunological mechanism. Serial studies of this type are needed in a larger number of patients to clarify this important point, which has implications for streptokinase dosage and also the duration of subsequent heparinization.

The dose of streptokinase was adjusted to maintain a thrombin time of 2-4 times a base-line value for pooled normal plasma rather than the base-line of the patients' own pretreatment thrombin times. The latter varied between 12 sec and 29 sec, probably because of raised serum F.D.P., and if streptokinase dosage is based on these values then the therapeutic effect and the degree of plasminogen depletion will vary from patient to patient. In four of our five patients the hourly streptokinase dose had to be decreased to 25-50% of the initial dose to maintain a thrombin time greater than twice the control value; this resulted in a maximal plasminogen depletion for four of the five children to between 3% and 7% of the pretreatment value.

There are, therefore, significant problems in using streptokinase in the haemolytic-uraemic syndrome and in being able to evaluate the effect. Its advantage over supportive therapy alone will need to be substantial before it can be widely advocated for use in paediatric units with little or no experience of thrombolytic therapy. Co-operative clinical trials are now needed and we suggest that these should contain a group of patients receiving supportive therapy alone; this will allow a prognostic index to be devised and will facilitate serial coagulation studies to determine whether the stimulus to intravascular coagulation still persists after admission.

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References

- Alkjaersig, N. (1960). In *Proceedings of the Conference on Thrombolytic Agents*, ed. H. R. Roberts and J. D. Geraty, p. 136. Chicago.
- Alkjaersig, N., Fletcher, A. P., and Sherry, S. (1959). *Journal of Clinical Investigation*, **38**, 1086.
- Ashton, E. H., and Flinn, R. M. (1969). *Abstracts Datafair 69*. British Computer Society, London.
- Bergstein, J. M., Edson, J. R., and Michael, A. F. (1972). *Lancet*, **1**, 448.
- Dacie, J. V., and Lewis, S. M. (1968). *Practical Haematology*. London, Churchill.
- Das, P. C., *et al.* (1967). *British Medical Journal*, **4**, 718.
- Gasser, C., *et al.* (1955). *Schweizerische medizinische Wochenschrift*, **85**, 905.

- Gianantonio, C. A., *et al.* (1968). *Journal of Pediatrics*, 72, 757.
 Gianantonio, C. A., *et al.* (1973). *Nephron*, 11, 174.
 Gilchrist, G. S., *et al.* (1969). *Lancet*, 1, 1123.
 Gower, J. C. (1966). *Biometrika*, 53, 325.
 Gower, J. C. (1968). *Biometrika*, 55, 582.
 Gower, J. C., and Ross, G. J. S. (1969). *Applied Statistics*, 18, 54.
 Habib, R., *et al.* (1969). *Archives Françaises de Pédiatrie*, 26, 391.
 Hardisty, R. M., and Ingram, G. I. C. (1965). *Bleeding Disorders*, Oxford, Blackwell Scientific.
 Kaplan, B. S., *et al.* (1971). *Journal of Pediatrics*, 78, 420.
 Katz, J., *et al.* (1971). *Journal of Pediatrics*, 78, 426.
 Katz, J., *et al.* (1973). *Journal of Pediatrics*, 83, 739.
 Lieberman, E. (1972). *Journal of Pediatrics*, 80, 1.
 Merskey, C., Kleiner, G. J., and Johnson, A. J. (1966). *Blood*, 28, 1.
 Metz, J. (1972). *British Journal of Haematology*, 23, Suppl., p. 53.
 Moncrieff, M. W., and Glasgow, E. F. (1970). *British Medical Journal*, 3, 188.
 Monnens, L. (1974). Personal communication.
 Monnens, L., *et al.* (1972). *Acta Helvetica Paediatrica*, 27, 45.
 Poliwooda, H., *et al.* (1969). *New England Journal of Medicine*, 280, 689.
 Prentice, C. R. M., and McNicol, G. P. (1973). In *Recent Advances in Thrombosis*, ed. L. Poller, p. 151. London, Churchill.
 Ratnoff, O. D., and Menzie, C. (1951). *Journal of Laboratory and Clinical Medicine*, 37, 316.
 Sanchez Avalos, J., *et al.* (1970). *Journal of Pediatrics*, 76, 538.
 Sharp, A. A., and Eggleton, M. J. (1963). *Journal of Clinical Pathology*, 16, 551.
 Sherry, S. (1973). *Thrombosis et Diathesis Haemorrhagica*, 29, 3.
 Shinton, N. K., *et al.* (1964). *Archives of Disease in Childhood*, 39, 455.
 Tune, B. M., Leavitt, T. J., and Gribble, T. J. (1973). *Journal of Pediatrics*, 82, 304.
 Vitacco, M., Sanchez Avalos, J., and Gianantonio, C. A. (1973). *Journal of Pediatrics*, 83, 271.
 Willoughby, M. L. N., *et al.* (1972). *Archives of Disease in Childhood*, 47, 766.
 Winterborn, M. H., White, R. H. R., and Stuart, J. (1972). *Lancet*, 1, 1071.

New Japanese Rubella Vaccine: Comparative Trials

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Summary

A total of 142 seronegative volunteers were given one of the following rubella vaccines: Cendehill, HPV77, DE-5, RA27/3, or a new Japanese vaccine, To-336. To-336 vaccine produced a slightly higher geometric mean antibody titre (G.M.T.) (65.7) than did the HPV77, DE-5 (63.1) or RA27/3 vaccine (61.9) but the G.M.T. induced by Cendehill vaccine was much lower (39.3).

Reactions, particularly joint symptoms, occurred least commonly after vaccination with To-336 vaccine. Joint symptoms occurred within seven days of menstruation in 30 out of 37 (81%) vaccinees ($P < 0.01$); their incidence was not related to oral contraception.

Though there is evidence to suggest that Japanese virus strains may be non-teratogenic further data on the incidence of congenitally acquired infection in Japan must be collected before this conclusion can be supported on epidemiological grounds.

Introduction

Vaccination with attenuated rubella virus now makes congenitally acquired rubella a potentially preventable disease, and in the U.K. it is official policy to offer vaccination to 11-14-year-old schoolgirls (Department of Health and Social Security, 1970). Though routine rubella vaccination of women of child-bearing age is not advised it is recommended for women at special risk of acquiring rubella—for example, nurses and schoolteachers (Department of Health and Social Security, 1972). Because the teratogenicity of the current attenuated vaccines is unknown, however, vaccinees must not become pregnant for at least two months after vaccination (Department of Health and Social Security, 1970). Despite this warning many terminations have been carried out because of inadvertent vaccination during pregnancy (Center for Disease Control, 1973; Registrar General, 1973). Furthermore, analysis of U.S. data shows that the im-

mune status of 86% of 208 pregnancies terminated for this reason had not been established before vaccination (Center for Disease Control, 1973). Thus vaccines composed of strains that are not transmitted to the fetus, provided that they are still immunogenic, would constitute a major advance in the development of rubella vaccines.

Some Japanese workers claim that though the pattern of postnatally acquired rubella in Japan is similar to that in most western countries congenitally acquired disease is rare, and they have attributed this to the fact that Japanese strains are of reduced teratogenicity (Kono *et al.*, 1969). In vitro studies have shown that when compared with U.S.-derived strains with a similar passage history Japanese strains induce significantly higher levels of interferon in primary human placental cell cultures but not in fetal lung cultures or leucocyte preparations (Banatvala *et al.*, 1971; Potter *et al.*, 1973). One of these Japanese strains, To-336, has been attenuated by passage in primary guinea-pig kidney cell cultures and undergone preliminary trials in Japan (R Kono, personal communication). Though encouraging these trials are difficult to evaluate, for the To-336 vaccine was not tested in parallel with established vaccines. We have therefore compared this new vaccine with RA27/3, Cendehill, and HPV77, DE-5 vaccines, the last of which, though extensively used in the U.S.A. and other parts of the world, is not yet licensed in the U.K.

Subjects and Methods

Sera from 578 nurses, 78 student physiotherapists, and 20 laboratory technicians at St. Thomas's Hospital aged between 17 and 32 years were screened for rubella antibodies by haemagglutination inhibition (H.A.I.) tests (Feldman, 1968; Cooper *et al.*, 1969). Altogether 150 (22.2%) were seronegative, 142 of whom participated in the trial. After signing a consent form each subject was allocated at random to one of four groups, each of which was allocated one of the following vaccines: Cendehill (Huygelen and Peetermans, 1967), HPV77, DE-5 (Buynak *et al.*, 1968), RA27/3 (Plotkin *et al.*, 1965), and To-336. All vaccines were given subcutaneously and the subjects were bled about eight weeks after vaccination. Sera taken before and after vaccination were tested in parallel by H.A.I. using four units of antigen.

Virus Excretion.—Whenever possible swabs were taken from 12 to 17 subjects in each group on alternate days between the 8th and 29th days after vaccination. Pharyngeal and nasal swabs taken on each occasion were immersed in the same bottle of 4 ml of virus transport medium. Specimens were inoculated into

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