Summary

The appearance of cerebral oedema following hypoxic episodes is described; its incidence appears to be more common than hitherto realized and may follow cerebral hypoxia from a variety of causes.

Dehydration therapy with 50% sucrose followed by 10% dextran is described: favourable results have been obtained from its use.

Some successful cases are reported.

The wider application of dehydration therapy is suggested; histopathological reports from the literature confirm that this would be rational.

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The Report of the Work of the London School of Hygiene and Tropical Medicine for the Year 1954-5 has recently been published. Considering the problem of the ethics of clinical trials, the report from the department of medical statistics and epidemiology stresses that experimental requirements are always subservient to the consideration of whether a treatment, beneficial but unproved, can be withheld from a patient, or "dummy" treatment given. Sometimes the length of a trial is not determined beforehand so that it can be stopped if significant differences appear in the results, though this may impose serious limitations on the researcher. Among new studies undertaken during the year the Ross Institute of Tropical Hygiene has for the first time opened research into the resistance of anophelines to insecticides as part of the world-wide drive against malaria, strongly supported by the recent World Health Assembly. The new concept of the total elimination of malaria wherever feasible ensures that the disease is likely "to take a large part of the interest of people concerned in tropical hygiene." The future policy of the school for 1957-62 must be submitted to the university at the end of the year. The one respect in which the school is cancelling development plans is at Winches Farm, used for breeding laboratory animals. In atomic warfare a wartime base so near to London would be valueless.

ANTIGENICITY STUDIES ON SALK-TYPE POLIOMYELITIS VACCINE

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The recent report from the United States Poliomyelitis Vaccine Evaluation Center (Francis *et al.*, 1955) on the efficacy of vaccine used in the 1954 field trials has encouraged hopes that effective control of paralytic poliomyelitis is now at hand. In early 1955 pilot quantities of vaccine were prepared in the United Kingdom. In May it was decided that the vaccine should not be used because it was prepared from the Mahoney Type 1 virus. However, one vaccine batch had already been used in a small number of human volunteers in the Sheffield area, and it is therefore of interest to compare the human response to immunization with the results of potency tests carried out previously on laboratory animals.

Technical Methods

Vaccine Preparation

In general, the methods used were similar to those of U.S. manufacturers, as outlined in the "Minimum Requirements for Poliomyelitis Vaccine" issued by the National Institutes of Health. Types 1, 2, and 3 poliomyelitis viruses were represented by strains Mahoney, MEF-1, and Saukett, respectively. Virus strains were propagated in large tissue cultures of kidney tissue from the rhesus monkey. After filtration, "strain pools" were inactivated with 1:4,000 formalin at 37° C. The trivalent preparation was formed by combining the inactivated single-strain pools in approximately equal quantities. Safety tests were carried out in tissue culture and on monkeys, and no active virus was detected. Merthiolate 1:10,000 was added as preservative. All potency tests were performed within four months of the date of manufacture.

Immunization Schedules

(i) *Mice*.—Mice in two groups of 40 were each given three injections of 0.5 ml. intraperitoneally at weekly intervals. For one group the vaccine was employed undiluted, for the other it was diluted 1:5. Ten days after the third injection 20 mice in each group were bled for determination of serum antibody titres in the pooled specimens. On the same day the remaining 20 mice in each group were used in a "challenge test" employing active MEF-1 virus inoculated intracerebrally (0.03 ml.). This test was performed to provide a direct measure of immunity, the challenge dose of virus being two hundred 50% mouse lethal doses (200 LD₅₀).

(ii) *Monkeys*.—Four normal rhesus monkeys were used; two received undiluted vaccine and two the vaccine diluted 1:10. Each animal received three inoculations of 1 ml. intramuscularly, the second dose being given one week after the first, and the third dose four weeks after the second. Monkeys were bled before the immunization course and two weeks after the final injection. Sera were tested in the tissue-culture neutralization test to determine antibody titres to the three virus types.

(iii) *Man*.—Fifteen volunteers from the medical staff and students at Sheffield University received vaccine. The

schedule was the same as that used for monkeys—three injections of 1 ml., administered at weeks 0, 1, and 5. The vaccine was given by the deep intramuscular route (deltoid) and was remarkably non-irritant. There were no local reactions, but one subject complained of abdominal pain and headache of three to four hours' duration, 12 hours after the first vaccine dose. It seems unlikely that this was a reaction to the inoculation. Initial serum specimens were collected about two to three months before the first injection. The subjects were bled again one week after the first injection and a final specimen was obtained two weeks after the third injection. All sera were stored at -20°C . until neutralization tests for antibody determination were carried out.

Tissue-culture Neutralization Tests

(i) *Glaxo Laboratories*.—These tests were performed in roller tube cultures of "trypsinized" monkey kidney tissue, as described by Youngner (1954). Synthetic mixture No. 199 (Morgan *et al.*, 1950) with 2% normal horse serum was used to initiate tissue growth. When confluent growth of epithelial cells had been achieved the medium was removed completely and 1.5 ml. of fresh medium without horse serum was added to each culture. Sera were tested in twofold dilutions over the range of the expected endpoint by inoculating the cultures with 0.5 ml. of the virus-serum mixtures. Standard virus pools representing each of the three virus types were employed at a dilution such that the inoculum per culture was approximately fifty 50% tissue-culture doses (50 TCD₅₀).

(ii) *Sheffield University*.—The only differences in technique were as follows: the nutrient medium employed consisted of calf serum (3 parts), lactalbumin hydrolysate (5 parts), and Hanks's balanced salt solution (92 parts); the sera were tested against 100 TCD₅₀ of virus instead of 50 TCD₅₀ and in fourfold dilution steps instead of twofold. In each laboratory appropriate virus and serum control tests were carried out whenever experimental sera were being titrated.

Results

Serum antibody titres in the groups of immunized mice and monkeys are shown in Table I. The response in mice was only moderate, the maximum titre produced being 1:32. There was considerable variation in the response of the

TABLE I.—Serological Response After Administration of Poliomyelitis Vaccine in Mice and Monkeys

Animal Species	Vaccine Dilution Inoculated	Reciprocals of Serum Antibody Titres* to Three Virus Types after Immunization		
		1 (Mahoney)	2 (MEF-1)	3 (Saukett)
Mice (white Swiss A2-G)	Undiluted	16	16	12
	Diluted 1:5	12	24	32
Rhesus monkeys	Undiluted	32 >256	96 128	<4 256
	Diluted 1:10	<4 256	8 >256	4 8

* All animals were initially antibody-free: antibody titres after immunization were determined on "pools" of serum from 20 mice and on individual specimens from monkeys.

monkeys, the titres ranging from <1:4 to >1:256. However, following immunization, antibody against each virus component of the vaccine was detected in animals of both species inoculated with either undiluted or diluted material.

The results of the challenge test in mice are illustrated in Fig. 1. Significant protection in the group which received undiluted vaccine (45% survival) was demonstrated, the rate for the group which received vaccine diluted 1:5 being 10%. Only 5% of the non-immunized control mice survived the challenge.

Table II shows the antibody titres determined for each virus type in the three serum specimens from each of the 15 human subjects inoculated. In Fig. 2 these results are

shown schematically. It will be noted that every subject showed a significant rise in antibody to at least one component of the vaccine.

It is interesting to compare the separate responses to each of the type components of the vaccine, and this comparison

FIG. 1.—Results of intracerebral challenge test with active MEF-1 (type 2) virus (200 LD₅₀) in groups of immunized mice. Vaccine was undiluted and diluted 1:5.

TABLE II.—Serological Response After Administration of Poliomyelitis Vaccine in Man

Reference No. of Subject	Virus Types for which Antibody Titre Determined	Reciprocals of Serum Antibody Titres		
		Initial Specimen	Intermediate Specimen	Final Specimen
1	1	<4	<4	128
	2	<4	8	128
	3	<4	<4	<4
2	1	<4	<4	64
	2	<4	<4	128
	3	<4	<4	16
3	1	<4	<4	16
	2	<4	<4	16
	3	<4	<4	<8
4	1	<4	<4	4
	2	<4	8	8
	3	32	>256	>256
5	1	<4	<4	8
	2	4	8	256
	3	128	>256	>256
6	1	32	>256	>256
	2	>256	256	256
	3	<4	4	256
7	1	32	128	256
	2	>256	>256	>256
	3	<4	16	128
8	1	>256	>256	>256
	2	<4	16	256
	3	>256	>256	>256
9	1	<4	16	128
	2	>256	>256	>256
	3	<4	<4	128
10	1	128	256	128
	2	<4	16	64
	3	32	>256	>256
11	1	128	>256	256
	2	<4	4	256
	3	<4	<4	128
12	1	16	>256	>256
	2	8	>256	256
	3	8	>256	>256
13	1	8	64	64
	2	64	256	>256
	3	4	128	>256
14	1	128	256	>256
	2	128	>256	128
	3	64	>256	>256
15	1	8	>256	256
	2	8	>256	256
	3	4	>256	>256

is best made in individuals without initial antibody to the type under consideration. Thus there were six subjects in whom Type 1 antibody was not detected in the first serum specimen. All had developed such antibody by the end of the vaccine course and the titres ranged from 1:4 to 1:128. All seven subjects in whom no Type 2 antibody was detected initially were found to have developed such antibody at the time of the third bleeding, and the titres ranged from 1:8 to 1:256. However, all but two of these subjects had antibody in the second serum specimen one week after the first injection of vaccine, suggesting that they had been previously sensitized to the antigen. Of the seven subjects without Type 3 antibody in the first serum specimen, five had not developed it at the time of the second bleeding, and in two antibody was still not detected on completion of the vaccine course.

FIG. 2.—Antibody titres in 15 adults inoculated with three virus types of vaccine.

FIG. 3.—Human response patterns following poliomyelitis immunization: classification on basis of prior immune status.

In addition, all subjects who possessed antibody for a particular virus type in the initial specimen showed a rapid and marked rise in titre after the first injection of vaccine, except in those subjects where the initial titre was at the upper limit of the range of the test and in whom, therefore, no further rise could be detected. Thus, in 36 of the 38 instances where it was technically possible to demonstrate an antibody rise, such a rise was in fact observed.

The differences in the post-immunization responses of individuals with and without circulating antibody to poliomyelitis viruses have been fully commented on by Salk (1955a, 1955b). It is interesting that these differences can be elicited from the limited data in this report. Thus the subjects can be divided into three main groups on the basis of antibody titres in the initial serum specimens. These are: (A) No antibody detectable for any virus type (subjects 1, 2, and 3 in Table II); (B) antibody present for one or two of the three types (subjects 4 to 11); (C) antibody present for all virus types (subjects 12 to 15). Fig. 3 depicts the mean geometric antibody titres for each group initially, after the first dose of vaccine, and on completion of the vaccine course. The "booster" effect after the first dose is demonstrated in Group C subjects, and in Group B subjects for those virus types for which antibody was present initially. It is also noteworthy that the response of the Group B subjects to the antigen for which no antibody was initially present was accelerated in comparison with the response of the subjects in Group A. This may indicate that previous experience of one virus type may "sensitize" the antibody production mechanism for heterologous types, and therefore that certain antigenic components may possibly be shared by the three virus types (Melnick, 1955).

It is concluded that the vaccine is an effective antigen for adult humans, although there is some suggestion that the Type 3 component in this particular batch was not as potent as the Type 1 and Type 2 components.

Discussion

Despite the small numbers of subjects in the tests, there is a broad measure of correlation between the serum antibody titres detected in monkeys and in human subjects of Group A—that is, individuals with no antibody to any virus type prior to vaccine inoculation. This correlation is also shown when the separate responses to each of the three type antigens are considered. As a guide, therefore, to the probable performance of a vaccine batch in man it seems that the monkey is a satisfactory laboratory animal. On the other hand, the antibody response in mice appears to be a less sensitive measure of vaccine potency. The results of the challenge test in mice, however, in which 45% of mice which had received undiluted vaccine survived an intracerebral inoculation of living Type 2 virus, indicate that the presence of only moderate serum antibody titres is associated with significant active immunity. It might be expected, therefore, that similar antibody levels in man would result in an even greater degree of protection, as in natural infections the virus reaches the central nervous system indirectly, after a stage of multiplication in the alimentary tract, rather than being introduced direct into the brain.

The data presented here show that vaccine prepared from viruses of American origin stimulated the production of antibody when injected into human subjects in this country. Such antibody should be effective against poliomyelitis strains prevalent in Britain, because sera from monkeys inoculated with these American strains neutralized both these and also virus strains isolated in Britain in 1954-5 to approximately the same titre (Wood—unpublished observations).

As it is important to be able to protect children from 1 year old onwards from the paralytic effects of poliomyelitis virus infections, we would emphasize that little is yet known of how children under 5 years of age respond to Salk-type vaccines.

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RELATIONSHIP OF CORONARY HEART DISEASE TO RESPIRATORY DISABILITY

INVESTIGATION OF A RANDOM SAMPLE OF COAL-WORKERS AGED 55-64 FROM A MINING VALLEY IN SOUTH WALES

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Increasing breathlessness on exertion is a common feature of coal-workers' pneumoconiosis (Fletcher, 1948). The number of men presenting for consultation with this symptom increases with age, both because the prevalence of pneumoconiosis in the mining community is related to years of exposure to coal dust (Cochrane *et al.*, 1952; Fletcher, 1955a), and because the presence of pneumoconiosis accentuates the normal impairment with age of the physiological indices of lung function (Gilson and Hugh-Jones, 1955). The prevalence of disabling pneumoconiosis is consequently higher in the older age groups, so that the assessment of breathlessness may be complicated by the coexistence of coronary heart disease or of generalized atherosclerosis (Lavenne and Belayew, 1955). It is therefore necessary to determine whether there is any causal relationship between them.

The prevalence of coronary heart disease has recently been studied in a random sample of coal-workers aged 55-64 (Cotes *et al.*, 1955; Thomas *et al.*, 1956), and the opportunity was taken to investigate pulmonary function, with a view to determining if coronary heart disease was associated with pulmonary disability. At the time it was uncertain whether or not there would be sufficient cases of coronary disease in the sample to justify the comparison; however, 18 cases, giving a 38% prevalence of clinically active and silent coronary heart disease, were found, together with a relatively high prevalence of respiratory disability, as had been anticipated from other surveys of this type (Higgins, 1956). It is believed that the tentative conclusions which have been drawn from the observed relationship between the two syndromes in this randomly selected group may be of value, if only in provoking further studies of this kind.

Method

The sample of 55 men was drawn from the 1,160 miners and ex-miners aged 55-64 known to be living in the Rhondda Fach at the time of a survey carried out in 1953 (Cochrane,

1954). Two of these men had died, two had left the district, and four declined to co-operate; the remaining 47 (1 in 25 of the original population and 93% of the men in the sample who were living in the valley) were investigated.

The prevalence of coronary heart disease was established by clinical examination and electrocardiography, using criteria which are described in detail elsewhere (Thomas *et al.*, 1956). Breathlessness was not taken into account.

Breathlessness was assessed, using the questions recommended by Fletcher (1952), and recorded on a five-point scale. A man in grade 1 has no undue breathlessness when compared with other people of the same age; in grades 2 and 3 he is unable to keep up with normal people of the same age walking uphill and on the flat respectively. In grade 4, walking on the level is limited by breathlessness to 200 yards, and in grade 5 breathlessness occurs on the least exertion.

"Bronchitis" was diagnosed in those men who fulfilled the arbitrary criteria that during the last three years they had had one or more chest illnesses associated with increased cough and/or sputum and wheeze or tightness in the chest. Pulmonary heart disease was diagnosed, using previously described criteria (Thomas, 1951). The radiological category of pneumoconiosis was determined on the International Classification (1950) by duplicate readings (Cochrane *et al.*, 1951; Fletcher, 1955b).

The maximum voluntary ventilation was estimated as a measure of ventilatory capacity from the mean of three or four readings of the three-quarters of a second timed vital capacity (Kennedy, 1953), using a spirometer fitted with an electronic timer (Gaensler, 1951). The results obtained with this indirect method correlate well with those obtained when the maximum voluntary ventilation is measured directly (Bernstein and Kazantzis, 1954; Kennedy, 1953). The ventilatory response to exercise at 300 kilogram-metres a minute, breathing oxygen, was obtained, using a standardized exercise step test (Hugh-Jones and Lambert, 1952).

The rise in blood lactic acid attributable to the exercise was determined as a measure of the adequacy of the blood supply to the active muscles, using a method previously described (Cotes, 1955).

Results

Bronchitis.—"Bronchitis" was diagnosed in 16 men (34% of the whole group). Of these, four reported one chest illness only; 12 more than one. Of the 16 men, 13 said that their sputum was yellow during their illnesses; three of them had not noticed the colour. All had a productive cough apart from their acute attacks, and at least those who had more than one such episode would probably be generally regarded as having some degree of chronic bronchitis. Two subjects with typical established chronic bronchitis had pulmonary heart disease.

Pneumoconiosis.—Twenty-eight subjects had pneumoconiosis with x-ray category 1 or above: 17 (36% of the sample studied) had some degree of simple pneumoconiosis, and 11 (23%) had progressive massive fibrosis. One of the latter had pulmonary heart disease.

Relationship between coronary heart disease, bronchitis, and pneumoconiosis.—The number of subjects with coronary heart disease, bronchitis, or progressive massive fibrosis who also had either of the other two conditions is given in Table I, together with the number of cases which would have been anticipated from their overall incidence on a proportional basis. The numbers are small, but they do not suggest any relationship between the three conditions.

Maximum voluntary ventilation in relation to clinical grade of breathlessness, coronary heart disease, bronchitis, and progressive massive fibrosis.—A significant relationship was found between the maximum voluntary ventilation and the clinical grade of breathlessness in the present series (Regression line, M.V.V. (indirect) = $108.5 - (17.07 \pm 2.247) \times \text{clinical grade}$). This relationship is comparable with that observed in a previous series, when the maximum voluntary