Antibody Response in Children with Protein Malnutrition

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IN A RECENT paper on "Infection and Kwashiorkor," Scrimshaw et al.[1] quoted twenty-four reports by workers in many parts of the world, all of whom mentioned the frequent association of kwashiorkor with various types of infection.

The precipitating effect of various infections on the development of kwashiorkor is well recognized.[1,2] Furthermore, in comparison with well nourished infants, these patients appear to have a reduced resistance to infection which is regarded as a major cause of death.[1,3]

Some excellent review articles have recently appeared on the interaction between malnutrition and infection[4,5] and, therefore, no attempt will be made to discuss this problem at any length. A reduced capacity to form antibodies in response to an antigenic stimulus in patients with kwashiorkor is an obvious possibility. In certain laboratory animals severe protein deficiency definitely causes a diminished antibody response.[6] The same applies to many vitamin deficiencies including those of pyridoxine, pantothenic acid and folic acid.[7]

In man, however, conflicting results have been obtained by different workers. Wohl et al.[8] found the capacity for antibody formation against typhoid vaccine to be reduced in adult patients with lowered serum albumin values caused by various chronic diseases. On the other hand Balch[9] found severely ill hypoproteinemic adults capable of producing antibodies against diphtheria toxoid as well as or even better than healthy control subjects. In Mexico[1] a retardation in the development of antibodies against diphtheria was found in malnourished infants, but in South Africa Kahn et al.[10] found no reduction of the isohemagglutinin titers of fourteen severely malnourished children.

In view of these conflicting reports we decided to investigate antibody response to typhoid vaccine in patients suffering from kwashiorkor. As pyridoxine deficiency seems to occur commonly in such patients in this area,[11] pyridoxine was administered intramuscularly to all the patients in one of the experimental groups.

MATERIALS AND METHODS

Experimental Groups

The investigation was carried out on thirty Bantu infants all of whom fulfilled the diagnostic criteria of kwashiorkor laid down by Brock and Autret.[12] On admission the patients were allocated at random to one or other of two experimental groups, each consisting of fifteen patients. The patients of both groups received a diet consisting of skimmed milk and ward diet, but one group received, in addition, 12.5 mg. pyridoxine daily by intramuscular injection. The patients of both groups received a daily oral supplement of a multivitamin mixture. During the first week of hospitalization all patients received 300,000 units of a penicillin V suspension orally every six hours. When indicated, broad spectrum antibiotics were also prescribed.

On the day following admission blood was taken from all patients for agglutination tests against H and O antigens of Salmonella typhi and for the determination of total serum proteins and individual serum protein fractions and for blood counts. Immediately after the blood was withdrawn, 0.1 ml. typhoid and paratyphoid A and B endotoxoid, manufactured by the South African Institute for

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TABLE I
Anti-O and H Titers* Before and After Typhoid Vaccination

Case No.	O Titer		H Titer	
	Initial	Final	Initial	Final
Patients with Kwashiorkor (without pyridoxine injections)				
1	...	100	...	25
2	...	800	...	200
3	50	200	...	...
4	50	200	...	100
5	...	1,600	...	1,600
6	...	400	...	100
7	...	200	...	200
8	...	1,600	50	200
9	...	800	...	400
10	...	400	...	...
11	...	400	...	400
12	...	...	...	...
13	25	400	...	200
14	...	1,600	...	400
15†	...	...	...	...
Patients with Kwashiorkor (with pyridoxine injections)				
16	...	1,600	...	1,600
17	...	800	...	200
18	25	400	25	200
19	...	...	50	50
20	...	800	50	100
21	...	400	...	400
22	...	400	...	100
23	...	800	...	200
24	...	400	...	200
25	...	25	...	...
26	...	200	...	...
27	...	400	...	3,200
28	...	...	...	...
29	...	...	...	Died
30	...	...	...	Died
Control Group				
31	...	1,600	...	400
32	...	800	...	400
33	...	...	...	...
34	25	800	...	100
35	50	400	...	100
36	...	400	...	200
37	...	800	...	200
38	...	800	...	200
39	...	400	...	200
40	...	800	...	200
41	...	800	...	800
42	...	800	25	400
43	25	800	...	400
44	...	800	50	100
45	...	1,600	...	800

* Figures represent the reciprocals of the highest dilutions showing agglutination.

† This patient died of bronchopneumonia after completion of the experiment.

Medical Research, was given intradermally. This amount was sufficient to produce antibodies but gave no severe local or constitutional reactions. Ten days later the blood tests were repeated.

Control Group

The control group consisted of fifteen recovered patients of good nutritional status. Ten of these patients (Cases 31 through 40; Table I) had recovered from kwashiorkor. With the exception of five patients (Cases 31 through 35) who were admitted one to one and one-half months before the tests were carried out, all patients had received the hospital diet for at least two months.

The remaining five patients, all of whom had been admitted at least a month previously, had initially suffered from various other diseases. At the time of the investigation, however, their health and nutritional state were excellent as judged clinically and also according to the serum protein values.

Laboratory Tests

The Widal tests was performed according to standard methods[13] used by the Institute for Pathology of the University of Pretoria. Total serum proteins were estimated according to the method of Weichselbaum.[14] Serum protein electrophoresis was carried out in an Eel paper electrophoresis apparatus with barbiturate buffer, ionic strength 0.1. Approximately 0.006 ml. serum was used for each estimation with a current of 2.5 ma. per strip. The strips were stained with 0.2 per cent bromphenol blue solution in 50 per cent ethanol saturated with mercuric chloride. The paper strips were cut and the individual protein fractions were eluted with 2 per cent soldium carbonate in 50 per cent methanol and read within thirty minutes in a Beckman model C colorimeter.

RESULTS

The results are set out in Tables I and II. Three patients died, two soon after admission and the third on the eleventh day after admission. As can be noted from Table I the majority of the patients with kwashiorkor seem to be able to produce antibodies against typhoid vaccine as efficiently as the healthy children in the control group. Statistical analysis of the final results by the U test of Wilcoxon, Mann and Whitney[15] did not show any significant differences between the three groups when compared two at a time ($P > 10$ per cent in all cases). For the purpose of sta-

TABLE II
Serum Protein Values: Means and Standard Deviations

Experimental Group	Total Proteins	Albumin	Alpha$_1$ Globulin	Alpha$_2$ Globulin	Beta Globulin	Gamma Globulin
Patients with kwashiorkor (without pyridoxine injections)	4.2 ± 0.87 (6.6 ± 0.94)	1.56 ± 0.38 (2.77 ± 1.01)	0.39 ± 0.10 (0.49 ± 0.10)	0.54 ± 0.13 (0.96 ± 0.28)	0.52 ± 0.16 (0.95 ± 0.22)	1.22 ± 0.46 (1.38 ± 0.48)
Patients with kwashiorkor (with pyridoxine injections)	4.3 ± 1.08 (6.9 ± 0.51)	1.66 ± 0.57 (3.30 ± 0.76)	0.38 ± 0.09 (0.50 ± 0.09)	0.55 ± 0.14 (0.88 ± 0.14)	0.47 ± 0.11 (0.94 ± 0.18)	1.26 ± 0.49 (1.24 ± 0.39)
Control group	7.3 ± 0.50 (7.5 ± 0.26)	3.67 ± 0.43 (3.87 ± 0.56)	0.45 ± 0.07 (0.50 ± 0.09)	0.88 ± 0.15 (0.84 ± 0.19)	0.92 ± 0.07 (0.88 ± 0.09)	1.34 ± 0.32 (1.39 ± 0.34)

NOTE: The first determination was obtained at the beginning of the experiment; the second (in parentheses) ten days later.

tistical analysis, only those patients who initially (before the injection of typhoid vaccine) showed a negative titer (<1:25) were included. Other patients were excluded, as an initially raised titer suggested a previous contact with salmonella infection or typhoid vaccine and the subsequent typhoid vaccination could be regarded as the administration of a "booster" dose.

In Table II are shown the average concentrations of the different serum protein fractions in the three groups of patients. The initial values and the increases obtained during the ten day period after the administration of typhoid vaccine were also analysed by means of the U test of Wilcoxon, Mann and Whitney. A probability level of $P < 1$ per cent was regarded as significant. No significant differences were found between the two groups of patients with kwashiorkor in respect to any of the serum protein fractions. Also no significant differences were found between the alpha- and gamma-globulin contents of the three groups tested, two at a time, either in respect to the initial values, or to the increases over the ten day period. The initial average values obtained for the various other protein components in both groups of patients with kwashiorkor differed significantly from the initial values for the patients in the control group ($P < 0.2$ per cent in all cases) confirming the well known observation that the low total serum protein values obtained in patients with kwashiorkor are largely due to depression of the serum albumin, alpha$_2$- and beta-globulin concentrations.

COMMENTS

The results obtained suggest that the capacity for antibody formation against typhoid vaccine by patients with kwashiorkor equals that of control subjects. These results appear to be at variance with those obtained in protein-depleted laboratory animals,[6] in hypoproteinemic adults[8] and in Mexican children with "third degree" malnutrition.[1] It should be kept in mind, however, that all our patients received high protein diets during the experimental period and, with the exception of the three patients who died, all responded well to treatment. The good response is reflected by the rapid rise in the serum albumin concentration and the alpha$_2$- and beta-globulin levels (Table II). Whether a satisfactory antibody response will occur if patients with kwashiorkor are kept on their "natural" maize diet is not known. Such an experiment, of course, is not justified and we are at a loss to know how to study this problem further. Although infection undoubtedly takes a heavy toll during the period of treatment of acute kwashiorkor, impaired antibody response does not appear to be an important factor in this respect.

Although, as can be seen from Table I, antibodies failed to develop in three patients with kwashiorkor after antigenic stimulation, a similar result was obtained in one control subject. It is of interest that the serum gamma-globulin levels of 0.73 and 0.6 gm. per 100 ml., respectively, found at the beginning and at the end of the experiment in Case 15, the only patient who died after completion of

the experimental period, are considerably lower than the average values shown in Table II. The gamma-globulin levels of the other patients who failed to show positive titers, however, are near the average value for the control subjects. In one patient, a fifteen year old malnourished girl, Krebs[16] found that a failure of antibody production against typhoid vaccine was correlated with a low level of gamma-globulin in the serum.

The possibility also exists that some of the subjects who comprised the control group, ten of whom had recovered from kwashiorkor, may not have been completely normal at the time of the experiment. Nevertheless, the antibody response of these ten subjects (Cases 31 through 40, Table I) equaled that of the five well nourished subjects (Cases 41 through 45) who had been admitted initially for treatment of other illnesses.

It has been shown in laboratory animals that pyridoxine deficiency is associated with diminished immunity response.[17] According to various chemical tests many of our patients seem to have suffered from a deficiency of this vitamin.[11] The administration of pyridoxine intramuscularly, however, did not result in more efficient production of antibodies or of gamma-globulin (Tables I and II).

The results obtained seem to be in agreement with those of Kahn et al.[10] who found no reduction of isohemagglutinins in severely malnourished children and who concluded that antibody production was not impaired in their patients. This is not altogether surprising as no reduction in serum gamma-globulin was encountered in the majority of our patients with kwashiorkor (Table II), a finding which corresponds to that of other workers.[18]

SUMMARY

The capacity for antibody production in response to typhoid vaccine was studied in thirty patients with kwashiorkor divided at random into two equal groups. Dietary treatment of the members of the two groups was identical except that the patients in one of the groups received, in addition, daily intramuscular injections of 12.5 mg. pyridoxine. A third group consisted of fifteen well nourished infants who served as controls. Immediately before the typhoid vaccine was administered on the day following admission and again ten days later, blood was taken for agglutination tests against "H" and "O" antigens of Salmonella typhi and for the determination of total serum proteins and individual protein fractions. No significant difference in the capacity for antibody production was found among the three groups.

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