Table 1. LYMPHOCYTE TRANSFORMATION IN RELATION TO THE TIME OF PREVIOUS VACCINATION

No.	Time from last vaccination	Humoral antibody titre: Haemagglutinin-inhibition test	Humoral antibody titre: Virus-neutralization test on CAM of chick embryos	Percentage of transformation at: 24 h	48 h	72 h	96 h	5 days	6 days	Maximum transformation in controls
1	50 yr	<10	<10	0·0	—*	0·0	0·0	0	—	0·2
2	50 yr	<20	<10	—	—	—	0·1	3·1	—	0·6
3	30 yr	Not tested		—	0·7	1·8	—	—	—	0·2
4	30 yr	<10	40	—	—	0·0	0·0	—	1·3	0·6
5	20 yr	Not tested		0·0	—		—	2·7	—	0·0
6	14 yr	<10	<10	1·7	2·2	—	4·9	6·3	—	0·9
7	13 yr	5	<10	—	—	1·2	—	3·8	—	1·4
8	6 yr	<10	<20	0·1	5·8	6·0	1·0	—	—	0·4
9	6 yr	<10	80	—	6·0	—	4·3	3·4	—	1·2
10	6 yr	5	40	—	—	—	9·8	6·0	7·1	0·6
11	3 yr	<10	160	—	3·3	—	—	—	—	0·2
12	5 months	10	40	—	1·7	—	8·6	—	—	0·4
13	1 month	<10	<20	13·1†	0·8	3·7	4·8	—	—	0·7
14	1 month	5	40	2·0†	1·8	1·8	7·4	22·2	—	1·1
15	1 month	<5	10	—	12·1	—	—	—	—	0·1
16	1 month	10	Not tested	5·8†	—	—	—	—	—	0·4
17	1 month	20	,, ,,	3·8†	7·4	—	—	—	—	0·1
18	Hyperimmune subjects	10	160	45·3†	—	27·8	—	10·7	6·4	1·0
19	,, ,,	<5	160	30·4†	4·6	—	16·8	—	—	1·4
20	,, ,,	<5	320	14·0†	—	—	—	—	—	0 8
21	,, ,,	10	160	7·6†	24·3	—	—	—	—	0·7

* Not tested. † Among transformed cells, elements of the intermediate type described in the text are predominant.

test is of no value in the determination of the intensity of immunity. The most similar and the highest virus neutralizing antibody titres (Nos. 18–21) were found in hyperimmune subjects who exhibited maximum transformation (with low titres of antihaemagglutinins). In this case there was a correlation of serological factor with intensity and speed of transformation. When the level of immunity is high some indices probably correlate with each other.

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Inhibition of the Macroglobulinaemic Phase of the Antibody Responses

REPEATED antigenic stimulation usually affects the immune response, qualitatively and quantitatively, by shifting the antibody equilibrium from the 19*S* towards the 7*S* class[1].

In the hope of understanding the underlying mechanisms involved in the immune response, we have assumed that a dose of antigen exists which, when administered repeatedly to laboratory animals from the day of birth, would specifically inhibit the macroglobulinaemic phase of the response. It should be possible to interfere only with the 19*S* immunoglobulin synthesis, because in the response to antigens 19*S* antibody is synthesized before 7*S* (ref. 2).

In order to test this hypothesis, groups of Swiss albino mice were injected with sheep erythrocytes (10^6–2×10^9, suspended in 0·05 ml. of a balanced salt solution) every fourth day from the day of birth. Injections were given into the orbital branch of the anterior facial vein or into the caudal vein, according to the age and the availability of the vessels. The mice were killed at regular 5 day intervals from the sixth to the forty-first day of life.

The agglutinating titre of the serum was assayed by standard methods and the number of splenic plaque-forming cells (PFC) was assayed by a modification[3] of a technique[4] which measures the 19*S* antibody forming cells.

Isolated and washed splenic cells were resuspended in Eagle's medium at a concentration of approximately $2 \times 10^4/\mu l$. Equal volumes (50 μl.) of cell suspensions and of a 0·5 per cent agar Noble (Difco) solution in Eagle's medium (42°–44° C) and 100 μl. of a 20 per cent suspension of sheep erythrocytes were poured, mixed and evenly spread on the surface of a microscope slide previously coated with 0·1 per cent agar Noble.

After the agar had set, the slides were incubated at 37° C for 120 min and then for another 30 min with added complement (1/15 guinea-pig serum). The plaques were counted with the aid of a hand magnifying glass and a strong indirect light source.

Results show that the agglutinating antibody titres and the number of plaque forming cells (PFC) do not vary for doses from 10^6 to 5×10^8 of sheep erythrocytes. A sudden change in the immune response occurs with a dose of 2×10^9 cells, when antibody production is so delayed that peak titres are reached 10 days later than they are with lower doses of sheep erythrocytes (Fig. 1), and the number of PFC remains at the basic value (Fig. 2). With lower doses of sheep erythrocytes, the number of PFC rises to 100 on the sixteenth day and then decreases slowly, reaching the basic value about 36 days after the start of the experiment.

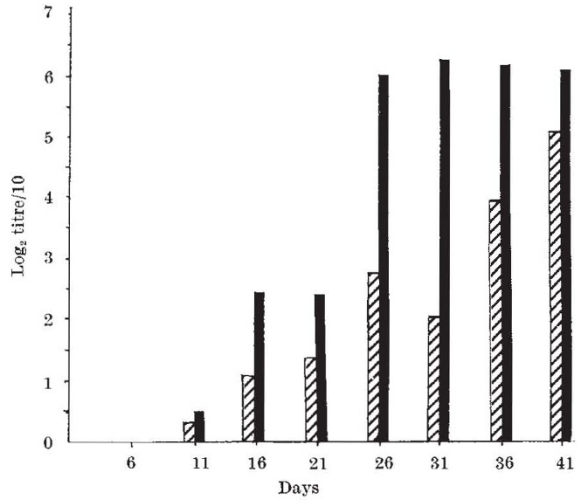

Fig. 1. Agglutinating titres from mice stimulated repeatedly with sheep erythrocytes. Log_2 of titres/10 are plotted against the age of animals (in days). Each point represents the mean of eight to ten mice. Black columns, 5×10^8; hatched columns, 2×10^9.

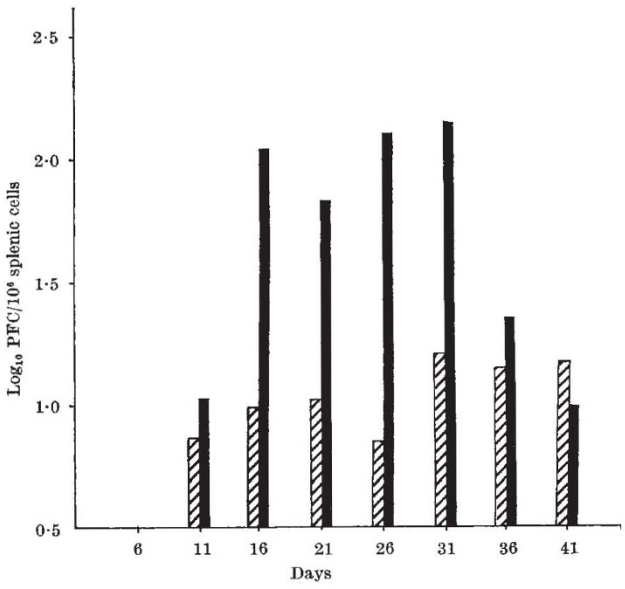

Fig. 2. Antibody formation by splenic cells from mice stimulated repeatedly with sheep erythrocytes. Log_{10} of PFC/10^6 spleen cells are plotted against the age of animals (in days). Each point represents the mean of eight to ten mice. Black columns, 5×10^8; hatched columns, 2×10^9.

The scarcity of PFC following a dose of 2×10^9 cells suggests that the circulating antibodies belong chiefly to the 7*S* class; the serum titre following treatment with 2-mercaptoethanol did not decrease compared with that of the unreduced samples.

In this system, it seems that the 2×10^9 dose of sheep erythrocytes represents a threshold at which the production of specific 19*S* macroglobulins may be inhibited, and at which the immune response consists of the synthesis of 7*S* antibodies. Although this response begins sluggishly, it later reaches levels similar to those induced by lower doses of cells.

This is the more remarkable in view of the fact that the erythrocyte antigens are usually able to induce a high and prolonged production of macroglobulin, even during the secondary and successive responses.

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Behavioural Measures of the Spectral Sensitivity of the Dark-adapted Goldfish

The eye of the goldfish has a single rod photopigment with a peak spectral absorption at about 522 nm, together with three different photopigments of the cones[1]. The spectral sensitivity curve of the electroretinogram (ERG) in the dark-adapted goldfish has been found to be considerably broader than the rod pigment absorption function, suggesting a significant contribution of cone response to the dark-adapted ERG[2]. This ERG result contrasts with those from very similar experiments on humans which show that the spectral sensitivity of the scotopic ERG is very closely related to the absorption spectrum of the visual pigment of the rods[3]. To elucidate more fully the relations between the psychophysics and physiology of goldfish vision, a behavioural method was devised to determine spectral sensitivity functions in fish. This method has previously been used to measure the spectral sensitivity of the goldfish in light-adapted conditions[4,5].

The apparatus was similar to that described previously[4] with some details changed. Two transparent 'Plexiglas' targets were suspended in the water at the front of a 3·5 gallon tank. Directly behind each of these targets, outside the tank, was a circular stimulus patch 13 mm in diameter. A third target was placed at the back of the tank. An electro-mechanical food delivery magazine was fixed above the centre of the experimental tank.

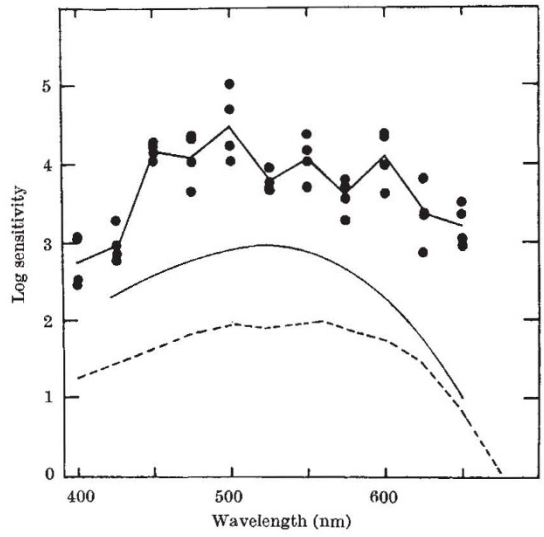

Fig. 1. Log $1/E_{7}\%$ (log relative spectral sensitivity) for two measurements on two dark-adapted subjects: the straight lines connect the means at each wavelength. The dashed function is the mean ERG relative spectral sensitivity for dark-adapted goldfish eyes from Burkhardt[2]. Data are in relative energy units at the cornea. Functions are displaced arbitrarily on the ordinate. The solid curve is the Dartnall nomogram for $\lambda max = 522$ nm.

Visual stimuli were provided by illuminating the translucent circular patches from behind with the grating image of a Bausch and Lomb monochromator. Stimulus wavelength and intensity were controlled by changing manually the monochromator setting and density filters between blocks of trials. No other light than that from the stimuli was present in the apparatus; subjects were able to find the food reinforcement in the absence of any light.

We used the common goldfish, *Carassius auratus*, about 7 inches long. Their home tanks were in a room lighted by four General Electric 200 W bottom silvered incandescent bulbs; the illuminance at tank level was 17 foot-candles. The room lights were turned off from 0600 h to 2400 h. Experimental sessions were started at about 1000 h, or after 4 h of dark-adaptation.

The full details of training procedures have been given before[4]. The basic experimental method was a two lever choice procedure with discrete trials. A response on the third lever was required to initiate a trial.

For measurement of spectral sensitivity, a press on the target at the rear of the tank exposed the stimulus light behind one of the transparent targets. In order to find the stimulus energy level of a given wavelength required for a