A good response to a fourth dose of the inactivated vaccine was found in most of the children.

No poliovirus inhibitors could be found in the faeces of these children either before or after the fourth dose.

A comparison was made of two different methods of titrating poliomyelitis antibodies.

Our thanks are due to the children who participated in this investigation and to their parents for permission to take serum samples.

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NUCLEAR CHANGES IN SQUAMOUS CELLS FROM BUCCAL MUCOSA IN PERNICIOUS ANAEMIA

BY

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In a previous communication evidence was briefly presented which confirmed the existence of abnormally large nuclei in oral squamous mucosa in pernicious anaemia (Farrant, 1958a). The present paper gives the results obtained by examining a series of patients with pernicious anaemia before and after treatment. In addition, further comparison of patients with pernicious anaemia and normal subjects has disclosed differences in nuclear shape and in variability of size.

Materials and Methods

Films of oral mucosa were obtained by scraping the inside of the right cheek with an ordinary microscope slide. The resulting material was spread on to a further slide, dried in air, fixed, and then stained by a standard technique (May-Grünwald-Giemsa). An image of the buccal squamous cells and their nuclei was projected by means of a microscope and a powerful source of light on to a ground-glass screen arranged so as to give a magnification of 1,000. Measurements were made in the centre only of this optical system in order to minimize the effects of spherical aberration. It was found difficult to measure the size of the squamous cells themselves because of their irregular outline. The squamous-cell nuclei, on the other hand, have a relatively uniform oval shape, and accordingly 100 nuclei in each preparation were examined and measured. The measurements made were those of the long and short axes. For each axis the means of these 100 measurements were used for comparison. Though variation in mean nuclear size among normal controls is considerable, it was found that the mean nuclear measurements in different parts of the same slide, and

different slides taken consecutively from the same person, varied by only a small amount.

Twenty-five patients with pernicious anaemia were examined both before and after treatment. Repeat films of oral mucosa were taken at intervals varying from 2 to 32 months after successful treatment. Criteria of diagnosis were the presence of a typical macrocytic blood film and of a megaloblastic marrow, the demonstration of a histamine-fast achlorhydria, and a satisfactory response to treatment with vitamin B₁₂ or liver. In eight patients these diagnostic criteria were incompletely fulfilled. Four patients had no bone-marrow examinations made, one died as the result of a fractured femur before treatment of her anaemia became effective, while the rest had either no test meals or test meals which showed achlorhydria but without histamine stimulation. The haemoglobin concentrations in 24 patients after treatment varied from 12.5 to 17.2 g./100 ml., with a mean of 14.4 g. Blood films were either normal or showed minor degrees of anisocytosis. One further patient had a haemoglobin value of 11.5 g./100 ml., but developed some changes of iron deficiency in the blood film.

Results

The mean values of the nuclear measurements have been compared for each axis, both before and after treatment, and the differences calculated (see Table).

Effect of Treatment Upon Mean Nuclear Size

Case No.	Long Axis in Microns			Short Axis in Microns		
	Before Treatment	After Treatment	Difference	Before Treatment	After Treatment	Difference
1	11.93	12.34	+0.41	9.32	9.47	+0.15
2	13.15	12.48	-0.67	10.50	9.01	-1.49
3	12.88	11.57	-1.31	10.30	8.78	-1.52
4	9.74	10.92	+1.18	6.87	7.12	+0.25
5	11.53	11.71	+0.18	9.13	8.48	-0.65
6	12.09	11.64	-0.45	9.83	9.35	-0.48
7	12.64	10.27	-2.37	10.16	7.48	-2.68
8	10.48	10.95	+0.47	7.95	7.98	+0.03
9	12.25	11.51	-0.74	9.81	8.40	-1.41
10	12.57	11.83	-0.74	9.74	8.90	-0.84
11	13.08	12.03	-1.05	10.58	9.19	-1.39
12	13.06	11.65	-1.41	9.78	8.32	-1.46
13	12.12	11.98	-0.14	9.48	8.99	-0.49
14	11.90	11.59	-0.31	9.52	8.60	-0.92
15	12.18	11.91	-0.27	9.41	8.83	-0.58
16	12.45	11.42	-1.03	9.55	8.82	-0.73
17	15.02	11.44	-3.58	11.50	8.85	-2.65
18	12.32	11.27	-1.05	9.76	8.62	-1.14
19	12.39	11.87	-0.52	9.13	9.07	-0.06
20	12.89	11.68	-1.21	10.43	8.89	-1.54
21	12.21	12.61	+0.40	9.73	9.61	-0.12
22	12.26	11.49	-0.77	9.64	8.50	-1.14
23	14.04	12.05	-1.99	11.52	9.19	-2.33
24	11.99	10.30	-1.69	9.60	7.72	-1.88
25	11.15	11.55	+0.40	8.33	8.51	+0.18

In six patients the mean values in one or other axis are greater after treatment than before, but only in four of them is this effect shown in the measurements of both axes, the other two showing a diminution in size of the short axis. In three of the four cases (Nos. 4, 8, and 25) normal values were obtained for both axes before treatment and one would therefore hardly expect any diminution in nuclear size after treatment. The fourth case (No. 1) showed only a minor degree of anaemia (12.1 g./100 ml.), and has been treated and maintained on an oral preparation of vitamin B₁₂. In all these four patients the increase in nuclear size after treatment is comparatively small. The remaining 19 patients all show varying degrees of diminution in nuclear size in both axes.

The results from these 25 patients with pernicious anaemia have been expressed for each axis as a frequency distribution curve. These show (Figs. 1 and 2) a highly significant decrease in nuclear size after treatment, the decrease being greater in the short axis. A population of larger nuclei among the untreated patients is also disclosed. Not only do the nuclei become smaller and more uniform in shape when specific treatment is given to a patient with pernicious anaemia, but in many cases the nuclear chromatin becomes more pyknotic and dense (Figs. 3 and 4).

This difference in the appearance of the nuclear material is reminiscent of the changes seen in the red-cell precursors in pernicious anaemia before and after

treatment. An experiment was therefore made to test the local effect of vitamin B₁₂ on the squamous-cell nuclei in untreated pernicious anaemia. Horrigan, Jarrold, and Vilter (1951) have already demonstrated an effect of local vitamin B₁₂ on the bone-marrow in pernicious anaemia. With suitable controls a very small dose of vitamin B₁₂ was injected into the oral mucosa of a patient with untreated pernicious anaemia, but no conclusive reduction in nuclear size was shown.

Nuclei of Abnormal Shape

In pernicious anaemia not only are the squamous-cell nuclei abnormally large but in addition they may have an irregular asymmetrical outline. In 28 out of 39 patients with pernicious anaemia previously examined abnormal nuclei were found, varying in incidence from 1 to 15 per 100 nuclei examined. The overall incidence in all patients was 3 per 100 nuclei. There was no correlation between mean nuclear size and the number of such abnormal nuclei observed. In contrast to these findings in untreated pernicious anaemia, 25 cases have been examined again after treatment, and among these, and among 62 normal subjects, only two abnormal nuclei were seen, one being found in each group.

Variability in Nuclear Size in Pernicious Anaemia and Normal Subjects

The values for the standard deviation in each set of 100 nuclear measurements have been calculated in 39 cases of pernicious anaemia, in 62 normal subjects, and in the 25 patients who have been studied both before and after treatment. The variability of nuclear size was significantly less among normal individuals and treated cases than among untreated cases of pernicious anaemia (Figs. 5 and 6). Thus not only are squamous-cell nuclei abnormally large in pernicious anaemia but their variation in size is greater than normal. Both values, those of mean and of standard deviation, revert towards normal with treatment.

Evidence has already been presented (Farrant, 1958b) that there is no effect of anaemia itself, of oral sepsis, or of achlorhydria in the absence of pernicious anaemia, on the size of squamous-cell nuclei from the mouth. Results among 95 normal subjects previously studied have been analysed according to the factors of sex, smoking, and, where relevant, position in the menstrual cycle. No association between any of these factors and squamous-cell nuclear size has been found.

Discussion

Relationship of Nuclear Changes to Disordered Metabolism in Pernicious Anaemia

It has been shown that nuclear abnormalities are found in oral epithelium in pernicious anaemia. The nature of these changes and their response to treatment

FIG. 1.—Effect of treatment on nuclear size in 25 cases of pernicious anaemia (long axis).

FIG. 2.—Effect of treatment on nuclear size in 25 cases of pernicious anaemia (short axis).

suggests a parallel with changes found in pernicious anaemia in the myeloid and erythroid tissues. There is much evidence for a disorder of nucleic-acid metabolism in this type of anaemia. Such a disorder may be responsible for changes in the red cells and white cells of bone-marrow and blood. Riddle (1929) has

suggested that purine and pyrimidine metabolism is abnormal in pernicious anaemia, and James and Abbott (1952), during a study of urinary phosphorus excretion, concluded that the reappearance of normoblastic erythropoiesis was accompanied by the rapid formation of nucleotides.

FIG. 3.—Pernicious anaemia (Case 17). Photomicrograph of oral mucosa before treatment. ($\times 570$.)

FIG. 4.—Pernicious anaemia (Case 17). Photomicrograph of oral mucosa four months after treatment. ($\times 570$.)

FIG. 5.—Comparison of standard deviation before (●) and after (○) treatment in 25 cases of pernicious anaemia.

FIG. 6.—Comparison of standard deviation in untreated pernicious anaemia and in normal controls. ▲ = P.A. ● = Controls.

Attempts to provide an explanation of the interaction of folic acid and vitamin B₁₂ in nuclear metabolism have been made by Vilter, Horrigan, Mueller, Jarrold, Vilter, Hawkins, and Seaman (1950) and by Nieweg, Faber, de Vries, and Kroese (1954). It has been suggested (Smith, 1958) that the action of vitamin B₁₂ on protein synthesis may occur by virtue of its effect on a number of enzyme reactions. It is not clear, however, why deficiency of vitamin B₁₂ should cause enlargement of certain cells and their nuclei. From a study of the relative amounts of deoxyribose nucleic acid and ribonucleic acid in vitamin B₁₂-deficient livers, Rose and Schweigert (1952) suggested that a reduced rate of nucleic-acid synthesis in turn reduced the rate of cell division, thus corroborating the hypothesis of Castle (1953) that the consequence of inability to divide was enlargement of cell and nucleus.

Abnormal Epithelial Cells and Nuclei in Pernicious Anaemia

Massey and Rubin (1954) have shown that the nuclei of gastric columnar cells in pernicious anaemia tend to be abnormally large, and that these changes are not altered by treatment. When studying the cytology of specimens obtained by gastric aspiration, Graham and Rheault (1954) observed that the cytoplasmic diameter of desquamated squamous cells in pernicious anaemia became smaller

after specific treatment. Boen (1957) has studied similar changes in mouth washings from cases of pernicious anaemia. A small series of patients and normal controls was studied, and the mean long diameter of the squamous-cell nucleus in certain of his cases was found to be abnormally large. Boddington (1959) has confirmed that enlargement of the nuclei from desquamated oral epithelial cells occurs in megaloblastic anaemia. More particularly this enlargement is related to epithelial atrophy of the tongue. Such a relation was not noted in this work (Farrant, 1958b), but the recognition of minor degrees of atrophy is difficult. In addition the epithelia studied may not be comparable.

In the present study variability of nuclear size in buccal epithelium has been found to be increased, and this is at least as characteristic a change as that of greater mean size in pernicious anaemia. In his study of the diameter of red blood cells in pernicious anaemia, Price-Jones (1933) found a correlation between variability of red-cell size and the number of red cells, and a less strict correlation between variability of size and haemoglobin level. He also noted that the mean size of the red cells, as opposed to their variability, bore no relation to the degree of anaemia. The present work has shown that the size of squamous-cell nuclei, too, is independent of the degree of anaemia.

The change to normal size and shape of the nuclei was observed to occupy only a few days once treatment in pernicious anaemia was started. Not infrequently a longer time may be needed for a smooth tongue to become normal. If enlargement of squamous-cell nuclei is a consequence of failure of normal nuclear division, and if changes in the basal layer of the tongue are similar in nature to those found in buccal epithelium, then impairment of growth of cells at the base of the filiform papillae might occur. This would allow desquamation of cornified cells from the tip of the papilla to exceed regeneration from below, and papillary atrophy would result. Conversely, when treatment began and normal cell divisions started, the need for increased regeneration to exceed continuing desquamation might account for the delay in the return to normal of the lingual epithelium, as compared with the changes caused by treatment which have been demonstrated in this study. Impaired activity of the basal cell layer of the tongue has been demonstrated in the glossitis of pernicious anaemia by Taft, Hughes, and Wood (1958).

In a few patients with untreated pernicious anaemia the squamous-cell nuclei have been found to be of normal size. Vitamin-B₁₂ deficiency may not always present in the same clinical form, and very low levels of serum vitamin B₁₂ have been reported (Mollin and Ross, 1954) in a small group of patients with subacute combined degeneration of the cord in the absence of significant anaemia. Sanford (1935), Brown (1946), and Bastrup-Madsen (1954) have all described the simultaneous occurrence of oral and neurological manifestations with little or no anaemia. The patients with squamous-cell nuclei of normal size did not, however, differ from the others with regard to the incidence of glossitis or subacute combined degeneration of the cord, and a cause for the presence in them of nuclei of normal size was not discovered.

Summary

By measuring squamous-cell nuclei from the mouth, abnormalities of nuclear size, shape, and variability of size have been demonstrated in pernicious anaemia. Treatment causes these abnormalities to regress. The possible significance of these findings is discussed.

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THE E.S.R. IN A NEW DRESS

BY

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The E.S.R. is one of the oldest investigations in clinical medicine, and appears to have developed in a crude form from observations on the blood in relation to "blood-letting." The first modern investigation of the diagnostic possibilities of the test was made by the Swedish physician Fåhræus (1921, 1929), who attempted to make use of a raised E.S.R. as a diagnostic test for pregnancy. He failed in his thesis, but developed a method of E.S.R. estimation that his pupil Westergren, working with male tuberculosis cases in Stockholm, was later to improve upon and publish in his classical paper in 1921.

Other methods have since been tried, notably that of Wintrobe (1933), but for various reasons, not discussed here, they have fallen from favour, and the Westergren method and its values still form the foundations for present-day clinical thinking.

The substance of this paper arose from the gradual evolution of hybrid methods for estimating the E.S.R. (or, more exactly, the erythrocyte aggregation sedimentation rate) as a result of laboratory modifications of the classical Westergren method without any