concentrations of $B_{12}$ of less than 30 pg/ml., whereas eight of the eleven non-smokers had concentrations greater than 25 pg/ml. If confirmed, this observation may be relevant in the elucidation of the pathogenesis of tobacco amblyopia.

The underlying reason for this investigation, apart from intrinsic interest, was the suggestion (mentioned by Phillips and McKenzie[4]) that the mechanism of tobacco amblyopia, or any toxic amblyopia, is a toxic effect on the retina of, say, cyanide or other toxin, which enters the eye with the aqueous humour and affects the retina, especially the macular area, from its vitreous surface. There is evidence for a flow of fluid from vitreous to retinal blood vessels and through retina to choroid[5]. This might explain why the retina is so selectively affected by certain toxins; the blood-aqueous barrier is not so high or so selective as the blood–retina barrier. Similar considerations might apply to the blood–cerebrospinal fluid barrier and blood–brain barrier. The response of the levels of aqueous $B_{12}$ to injected hydroxocobalamin and cyanocobalamin is being studied, together with thiocyanate levels.

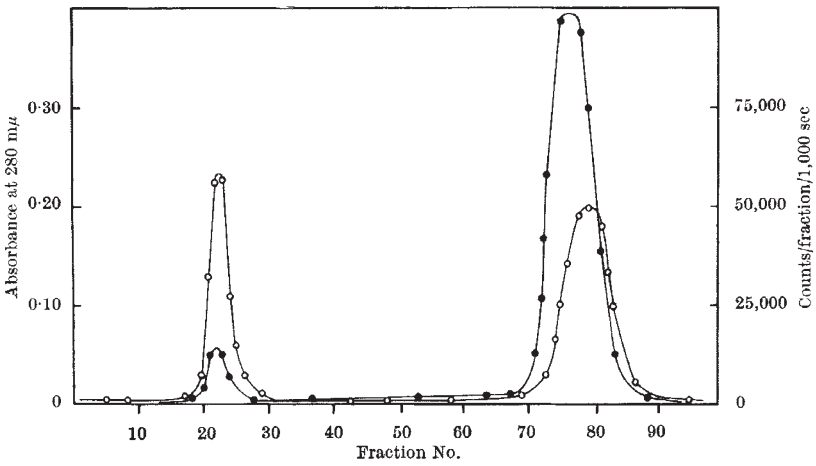


Fig. 1. Filtration on 'Sephadex *G*-200' of whole human saliva labelled with iron-59. Sample volume was 3 ml. Column dimensions were 2·5 × 40 cm. Buffer, physiological saline buffered to *p*H 8·0 with 0·02 molar HCl/$NH_4OH$. Fractions (3·5 ml.) were collected at a flow rate of about 30 ml./h. ○, UV absorbance; ●, radioactivity.

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## Iron Binding Properties of Saliva

Gastric juice of healthy people contains a high molecular weight iron binding protein "gastroferrin"[1]. A role for the gastric iron binding protein in the regulation of iron absorption has been proposed[2,3], and alterations in its production have been described in clinical conditions of iron deficiency[3] and iron overload[2]. The gastric juice obtained for the studies previously reported was free of saliva. Normally, however, swallowed saliva contributes substantially to the total volume of secretions present in the stomach. It was therefore decided to investigate the possible iron binding properties of this secretion. The effect of acid-peptic digestion on the iron binding properties of saliva was also studied because of the possibility that hydrochloric acid and pepsin secreted by the gastric mucosa react with the saliva present in the stomach.

Whole saliva was collected from thirty-five normal subjects who expectorated into a beaker. Saliva was found to possess marked iron binding ability when this was measured by a standard iron-59 solubility test previously described[4]. Iron binding capacity varied from 0·03 to 0·15 mg of $Fe^{+++}$/ml. of saliva (mean 0·08 mg of $Fe^{+++}$/ml.).

In the next set of experiments, saliva which had been labelled with iron-59 was resolved by molecular sieving through a column of 'Sephadex *G*-200'. The relative protein content of the eluted fractions was determined by optical density measurements at 280 mμ and the iron content by measurement of the gamma activity of the iron-59 label. A constant elution pattern of two protein peaks was obtained, a totally excluded peak of molecular weight greater than 200,000 and a peak of lower molecular weight. Both peaks contained bound iron-59 (Fig. 1). In contrast, iron binding in gastric juice occurs only in the high molecular weight fraction[1].

When saliva was labelled with iron-59 and subjected to digestion with 0·02 per cent w/v pepsin in 0·1 molar HCl for 1 h at 37·5° C, the protein content of the high molecular weight fraction was almost abolished. The absolute amount of iron-59 bound to this fraction was, however, not significantly altered. The high molecular weight iron binding substance in saliva is therefore resistant to acid-peptic digestion. The second fraction of lower molecular weight increased after acid-peptic digestion and also retained its iron binding activity.

Experiments with polyacrylamide disc gel electrophoresis[5] of saliva labelled with iron-59 using standard 7·5 per cent polyacrylamide gel columns and *tris*-glycine buffer, *p*H 8·3, showed that iron binding occurred in the slow moving components. These components have been shown by other workers to contain glycoproteins[6].

The total amount of high molecular weight iron binding substance secreted in saliva in 24 h is sufficient to bind approximately one tenth as much iron as can be bound by the estimated 24 h secretion of gastroferrin. The high molecular weight iron binding substance in saliva might have a function in health and disease similar to that of gastroferrin, both because of its molecular weight and because it is resistant to acid-peptic digestion.

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