Enzyme activity in the guts of earthworms Mean ± SEM

	L. mauritii				*O. surensis*				*D. calebi*				*D. bolaui*
	Ant.	Mid.	Post.	Av.	Ant.	Mid.	Post.	Av.	Ant.	Mid.	Post.	Av.	
Protein (mg)	1.06 ±0.22	0.68 ±0.14	0.59 ±0.12	0.78 ±0.10	0.58 ±0.23	0.46 ±0.08	0.17 ±0.11	0.40 ±0.09	0.38 ±0.03	0.19 ±0.02	0.14 ±0.03	0.24 ±0.03	0.14 ±0.03
Protease*	23.8 ±7.96	32.28 ±13.58	7.71 ±2.40	21.26 ±5.56	12.16 ±3.60	28.69 ±13.25	57.68 ±15.59	32.86 ±8.15	7.88 ±0.84	8.56 ±0.74	10.67 ±0.87	9.04 ±0.56	32.89 ±3.49
Amylase*	75.49 ±6.31	142.45 ±29.42	135.5 ±27.57	117.81 ±14.28	108.38 ±44.69	202.25 ±56.68	284.19 ±112.04	198.27 ±45.39	30.99 ±9.33	43.93 ±17.81	59.68 ±22.67	44.08 ±9.99	258.54 ±60.02
Invertase*	37.98 ±19.04	32.05 ±15.99	41.47 ±8.73	33.84 ±8.28	123.55 ±15.79	209.09 ±18.79	389.05 ±21.64	240.85 ±31.23	36.38 ±8.16	119.18 ±27.23	29.83 ±1.97	61.79 ±14.97	137.01 ±30.53
Cellulase*	16.66 ±5.30	29.48 ±3.51	37.39 ±9.46	27.84 ±4.13	42.10 ±18.82	78.95 ±9.15	119.83 ±19.03	87.69 ±10.75	12.51 ±2.03	20.98 ±3.28	37.76 ±5.31	23.75 ±3.73	141.36 ±29.79
Urease*	0.96 ±0.24	0.77 ±0.29	0.87 ±0.17	0.87 ±0.14	NA	NA	NA	NA	0.22 ±0.09	0.17 ±0.04	0.29 ±0.09	0.23 ±0.04	0.73 ±0.14

*µg/mg protein/h. NA, no activity; Ant., anterior region; Mid., middle region; Post., posterior region; Av., average.

O. surensis. Protease and cellulase activity are maximal in the posterior region of *O. surensis* and of *D. calebi* and in the middle region of *L. mauritii*. On the average, *O. surensis* shows maximum activity and *D. calebi* shows minimum activity for all the enzymes in the gut. Cellulase activity in whole tissue of *D. bolaui* is maximal compared with the gut enzyme activity of the above species. The differential enzyme-activity is perhaps related to the type of food and rate of eating of each species. In *Lumbricus terrestris* cellulase activity in the anterior half of the gut is 10-fold higher than in the posterior half[7] and the mucosa of crop, gizzard and fore-intestine (up to a portion corresponding to the 60th segment) secrete proteolytic enzymes[6]. The activity decreases sharply in the mucosa of the middle and hind intestine. Nielsen[10] observed on the basis of his qualitative study that of the 3 species of earthworms only *Dendrobaena octaedra* showed evidence of digesting cellulose and chitin and the other 2 species did not have the requisite enzymes to digest cellulose, pectin, xylan and chitin. The question whether cellulase present in various animals is produced by the gut flora or by the animals themselves is not very important from the ecological point of view as long as the possible association between animals and cellulotypic microbes is constant[10]. The finding that cellulase activity is maximal in the posterior region of the gut of the worms supports the view that microorganisms present in the fore and mid gut might be helping in the partial digestion and processing of the complex plant remains containing cellulose, xylan, mannan, pectin etc. This study indicates that tropical earthworms play an active part in the decomposition process in forest and grassland litter as they possess cellulase.

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Removal of polyethylene glycols from immunoglobulin samples by adsorption chromatography on polystyrene beads

B. Skoog

Kabi AB, Research Department, Analytical Chemistry, S-112 87 Stockholm (Sweden), 31 January 1980

Summary. A chromatographic method is described for 80% removal of polyethylene glycol of different types, 600–6000, from human immunoglobulin preparations. Bio-beads SM-2 were used in batch procedures or packed on a 16 × 100 mm column. The polyethylene glycols were desorbed with 75% ethanol.

The use of high molecular weight polyethylene glycols (PEG) for the fractionation and purification of proteins from human plasma, as an alternative to cold ethanol precipitation techniques, is now well documented[1–5]. However, no reliable method has yet been described for effective removal of the trace contaminating polymers in such protein preparations. When preparing plasma proteins by PEG fractionation procedures the remaining traces of polymers are now of the order of 0.15% (w/v). In gel filtration, for example, the linear polyethylene glycols behave like molecules much larger than expected[6,7]. Because of this, the effective hydrodynamic radii of the

polymers make gel filtration unsuitable as a separation tool.

However, the non-ionic surfactant Triton X-100 has been removed effectively from protein samples by chromatography on a polystyrene resin, Amberlite XAD[8,9] and on a rigid styrene-divinylbenzene matrix Bio-beads SM-2[10]. This effect has been explained in terms of the adsoprtion of the hydrophobic domain of Triton X-100 on the matrices. Since polyethylene glycol serves structurally as a part of different amphiphilic molecules, such as the polyoxyethylene p-t-octyl phenols, the Triton X series[11], it was tempting to adapt the method of Holloway[10] for the removal of polyethylene glycols. This report describes a novel chromatographic procedure for the removal of PEG contaminating human immunoglobulin G (IgG) preparations.

Material and methods. Polyethylene glycols, varying in mean mol.wts from 600 to 6000, were obtained from Merck AG, Darmstadt, FRG. Human serum immunoglobulin was prepared by the modified Cohn procedure used at Kabi AB[12]. All chemicals used were of analytical reagent grade and also purchased from Merck AG.

Immunoglobulin determinations were performed spectrophotometrically, using the absorptivity value ($A_{280}^{1\%}$) of 13.7, determined in this investigation. The absorption coefficient of highly purified human IgG was calculated from absorbance measurements at 280 nm (1-cm cell) in 0.1 mole/l phosphate buffer, pH 7.4. The protein content of the solution was determined by the biuret and micro-Kjeldahl methods. Determinations of polyethylene glycols were made by the barium iodide complex formation method[13]. The applied types and batches of PEG were used as standards in the determinations.

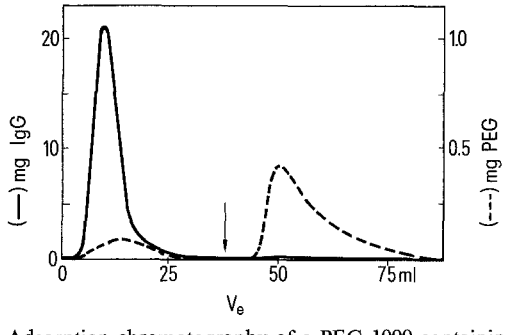

Fig. 1. Adsorption chromatography of a PEG 1000-containing IgG sample. 1.0 ml of a mixture containing 0.1% (w/v) PEG 1000 and 3% (w/v) IgG was applied to a 16×100 mm column and eluted with 10 mmoles/l phosphate buffer. The desorption with 75% ethanol was started at the point, indicated by the arrow.

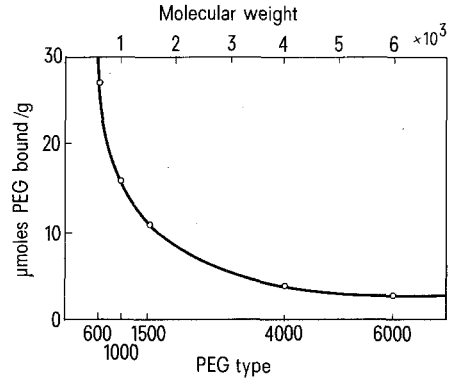

Fig. 2. The molar binding capacity of different PEG types to Bio-beads SM-2.

The styrene-divinylbenzene copolymer, Bio-beads SM-2 (Bio-Rad Laboratories, Richmond, California), was prepared according to Holloway[10] and used in batch procedures or in adsorption chromatographic runs packed in a 16×100 mm column (Pharmacia Fine Chemicals, Uppsala Sweden). Adsorption chromatography of polyethylene glycol samples alone or mixed with human IgG was carried out in 10 mmoles/l phosphate buffer, pH 7.2. 1.0-ml samples were applied to the column at a flow rate of 20 ml/h. Desorptions were carried out with an approximate ethanol concentration of 75%, established by gradient elution of the polyethylene glycols. After desorption the column was re-equilibrated with 10 mmoles/l phosphate buffer (pH 7.2) for a new cycle.

Results and discussion. An example of the adsorption and desorption profiles obtained when a PEG 1000-containing IgG sample was run on the Bio-beads SM-2 column described is shown in figure 1. PEG types of other mean molecular weights lower than 1000 were adsorbed in a similar manner. By the procedure described the PEG types applied were reduced by 80±2% in the IgG preparations. Because of the low exclusion limit of the Bio-beads SM-2, the adsorption was achieved without notable dilution. The IgG content of the samples was reduced by only about 4% during the adsorption. This protein was detectable after desorption.

The binding capacity of PEG to the Bio-beads SM-2 was estimated from the batch procedure to be 15.9±0.5 mg PEG/g. The adsorption of PEG to Bio-beads SM-2 increased with decreasing mean molecular weight (figure 2). This indicates that the oxyethylene groups of PEG are involved in the adsorption. The variation in the binding of PEG to Bio-beads SM-2 also depends on the nonhomogeneity of PEG with regard to its molecular weight. The actual molecular weight may vary 10–20% around the mean in 1 PEG type[1]. The addition of ethanol to the column causes disruption of the bonds and the matrix is regenerated.

Polystyrene beads, as used for chromatography, have the advantages of extreme strength and rigidity, which permits high flow rates and fast chromatographic runs. The disadvantage of the matrix is the high cost when used on a larger scale. Bio-beads SM-2 may therefore be replaced by similar materials based on styrene and its derivatives as pointed out by Cheetham[9]. Furthermore, the method described may be adopted for the removal of PEG in other contaminated human plasma protein preparations since the adsorption of most plasma proteins to Bio-beads SM-2 has been found to be low[14].

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