In vivo and in vitro Effect of Sulfathiazole on Serum Glycoproteins

Studying the effects of alimentary lipaemia, we observed[1] that the neutral carbohydrate content of albumin and β-globulin decreased while that of α-1, α-2 and γ-globulins increased. These modifications may be explained as due to a possibility of a physico-chemical competition between the lipides and carbohydrates for the transport surfaces of plasma proteins. An essential condition of such a competition is the unstable binding of some carbohydrate components by the carrier proteins. The purpose of this study is to obtain new data regarding the nature of carbohydrate-protein interactions by modifying the binding capacity of plasma proteins by sulphathiazole.

In vitro effect of sulphathiazole: Examinations were performed on 12 sera. 2 mg of sulphathiazole was dissolved in 1 ml of serum, the mixture was incubated for 1 h at 37 °C. The paper electrophoretic glycoprotein fractions were determined from the original sera and from the sera treated with sulphathiazole (Whatman 1 paper, barbital buffer at pH 8.6 and 0.05 ionic strength, PAS method, automatic scanning of the strips). The presence of sulphathiazole does not influence the intensity of the PAS reaction.

In vivo effect of sulphathiazole: Fourteen patients suffering from chronic diseases were examined. Before and after 1 h following the i.v. injection of sulphathiazole (0.02 g/kg) we determined the electrophoretic patterns of the glycoprotein fractions.

The statistical evaluation of the results was performed using Student's t test. The results are summarized in the Table.

	Glycoprotein fractions % Before sulphathiazole a.m.	s.e.m.	After sulphathiazole a.m.	s.e.m.	$P <$
In vitro					
Albumin	6.7	± 1.1	3.7	± 0.4	0.01
α-1 globulins	17.6	± 1.4	14.9	± 1.5	0.01
α-2 globulins	33.2	± 1.4	35.6	± 1.3	0.001
β-globulins	27.9	± 1.8	30.0	± 2.3	0.05
γ-globulins	14.6	± 1.5	15.8	± 1.7	0.50
In vivo					
Albumin	6.8	± 1.6	3.4	± 0.9	0.01
α-1 globulins	16.5	± 1.2	13.7	± 0.9	0.01
α-2 globulins	29.2	± 1.7	33.1	± 2.5	0.02
β-globulins	28.2	± 1.4	29.7	± 1.5	0.30
γ-globulins	19.3	± 1.2	20.1	± 1.5	0.40

a.m., arithmetical mean; s.e.m., standard error of the mean.

The statistical evaluation of the results was performed using Student's t test. The results are summarized in the Table.

In 1938 BENNHOLD[2] demonstrated for the first time the prontosil binding-capacity of albumin. Subsequently a number of authors enlarged our knowledge of the protein-sulphamide interactions. Recently, CLAUSEN[3] separated the sulphonamide-binding proteins by gel filtration on Sefadex column and pointed out that 71.5% of the sulphamide was bound by prealbumin, albumin and α-1 glycoprotein fraction; 5.6% by α-2 macroglobulin and 22.6% by non-antigenic polypeptides.

The lowering of the carbohydrate content of sulphamide carrier proteins, a phenomenon observed after the i.v injection of sulphathiazole as well as after its dissolution in serum, shows that some carbohydrate fractions are bound to proteins only by physico-chemical forces (van der Waals' and hydrophobic interactions, hydrogen bonding). This ensures a considerable variability for the surface of glycoproteins. The variability of the neutral glucide content of electrophoretic glycoprotein fractions is not in accordance with the opinion that all carbohydrate in glycoproteins is firmly bound to the intact protein[4]. This contradiction points to the unsatisfactory delimitation of the glycoprotein notion.

Zusammenfassung. Durch Sulfathiazolzugabe in vitro oder durch Sulfathiazolbehandlung in vivo wird der Glycoproteingehalt des Albumins und der α-1-Globuline reduziert.

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Effects of X-Irradiation on Ribonucleases in Blood Plasma

Enzymes capable of degrading RNA are present in blood[1,2], and in humans the alkaline RNAse activity is divided almost equally between the cells and the plasma[2]. An inhibitor of this enzyme is also present in erythrocytes[2].

RNAse levels in various tissues show large increases after irradiation[3–5]; in the case of alkaline RNAse these appear to at least partially result from the increased enzyme synthesis induced by a neuro-endocrine reaction stimulated by the stressing action of ionising radiation[5,6]. It was therefore decided to study the effects of X-irradiation on the RNAse levels in the plasma of rats and guinea-pigs, in order to examine whether the changed enzyme levels in tissues were accompanied by changes in plasma enzyme levels, with which they could be correlated.

Acid and alkaline RNAse activities were estimated by measuring the rate of degradation of RNA to acid-soluble nucleotides at pH 5.6 and 7.7 respectively, as described previously[6,7]. The animals were irradiated with 700 R of 250 KV_p X-rays filtered through 2 mm copper, and killed 18 h later; blood was taken from the heart by syringe. The control rats were sham-irradiated.

There was no detectable amount of the inhibitor of alkaline RNAse activity in plasma, but after haemolysis

considerable inhibitor activity occurred, as has been reported by GUPTA et al.[2].

Both acid and alkaline RNAses were present in plasma, and 18 h after whole-body irradiation the alkaline RNAse activity was increased by a factor of 2 in guinea-pig plasma, and the acid RNAse activity by a factor of 3.1 (Table). Present work on the radiation-induced variations in the RNAses of guinea-pig tissues may enable the relationships between the tissue and plasma enzyme levels to be clarified.

In rat plasma, the acid and alkaline RNAse activities were increased by factors of 1.6 and 7 respectively after irradiation (Table): in rat tissues, the acid RNAse levels also show smaller increases than alkaline RNAse levels[3-5]. The changes in plasma enzyme levels were presumably caused by a combination of (1) increased RNAse synthesis and (2) the release of the enzyme from damaged cells. It is possible to separate these 2 factors to some extent in rats, for work on spleen, thymus and intestine has shown that in these organs there appears to be increased alkaline RNAse synthesis and comparatively little cell damage after local-irradiation of the head (with the trunk shielded), while after local-irradiation of the trunk (with the head shielded) there is little change in RNAse synthesis but extensive cell damage[4-6]. Irradiation of the head (Table) caused the plasma alkaline RNAse level in rats to increase by a factor of 3.3, while irradiation of the trunk produced an increase by a factor of 1.8. The increase by a factor of 7 in the alkaline RNAse level of rat plasma after whole-body irradiation may therefore be the result of 2 effects: the increased rate of synthesis of the enzyme from damaged tissues into the blood, more than trebling the plasma RNAse level; and the increased rate of release of the enzyme from damaged tissues into the blood, doubling the plasma RNAse level.

Irradiation of the trunk and of the head of rats produced increases by factors of 1.2 and 1.3 respectively in the plasma acid RNAse level in rats (Table), but the significance of this cannot be determined, as the effects of localized irradiation on the acid RNAse levels in different tissues are not yet known.

Effects of whole-body and local-irradiation on plasma RNAse levels

Animal	Treatment	Acid RNAse activity[a]	Alkaline RNAse activity[a]
Guinea-pig	Control	4.95 ± 0.2[b]	7.4 ± 0.5[b]
Guinea-pig	700r whole-body irradiation	14.9 ± 1.0	14.8 ± 0.8
Rat	Control	2.9 ± 0.25	4.35 ± 0.3
Rat	700r whole-body irradiation	4.6 ± 0.2	30.4 ± 3.7
Rat	700r to head	3.8 ± 0.3	15.0 ± 1.3
Rat	700r to trunk	3.6 ± 0.2	8.3 ± 0.7

[a] μmole ribonucleotide liberated/h/ml of plasma. [b] Each value is the mean of the enzyme levels in 6 animals, the standard deviation of the mean is also shown.

Zusammenfassung. Unter Bestrahlung wurde die RNAse-Aktivität im Blut untersucht. Es zeigte sich, dass Bestrahlung des Kopfes allein zu einer starken Erhöhung der alkalischen RNAse im Serum führt.

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Anthocyanins in *Dioscorea alata* L.

Because of the recent paper of the same title by RAŠPER and COURSEY[1], we were prompted to publish our own results on the near total structure of the anthocyanins which make up the pigmentation of the West Indian variety 'St. Vincent Red' of *Dioscorea alata* L.

The standard methods of HARBORNE[2] were used to extract the pigments from the tubers of 'St. Vincent Red' yams so as to minimize the possibility of deacylation or other degradation of the dissolved anthocyanins. The pigments were then precipitated from the concentrated extract with dry ether, redissolved in methanol containing 0.5% HCl, and purified by repeated chromatography on Whatman 3 MM. paper. Banding in BAW – *n*-butanol-acetic acid-water (4:1:5 v/v) – produced 1 main pigment A, and 2 minor pigments B and C. The 3 pigments were rebanded in BAW and then in 15% HA – acetic acid-water (85:15 v/v) – to remove free sugars[3]. The minor pigment C, which was present in very small amount, faded in the aqueous solvent and could only be successfully rebanded in alcoholic solvents. The pigments were then eluted for structural analysis.

All 3 anthocyanins yielded a single anthocyanidin as the result of exhaustive acid hydrolysis. This anthocyanidin was magenta in visible light and bright pink in UV-light, and when spotted on Whatman No. 1 paper together with reference anthocyanidins, it had Rf values similar to cyanidin. This was confirmed by co-chromatography with an authentic sample of cyanidin and by a spectral analysis.

The UV-spectrum of the anthocyanidin in 0.01% HCl in methanol showed maxima at 535 and 280 nm and was identical with that of a solution of cyanidin prepared in the same way.

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