CHEMICAL STUDIES IN HYPERTENSION. REDUCING AND NITROGENOUS FRACTIONS IN PROTEIN-FREE BLOOD FILTRATES

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The literature concerning non-glucose reducing substances in the blood in clinical states involving vascular hypertension has apparently been limited to observations incidental to other problems. In 1925, certain investigators (1) found some increase in the non-glucose reducing fraction of zinc hydroxide (Hagedorn-Jensen) blood filtrates from patients with glomerulonephritis, some of whom presumably had hypertension. In 1927, Somogyi (2) noted an increase of reducing non-sugars in tungstic acid filtrates from patients with high nitrogen retention. Other authors (3), in 1929, reported significant amounts of non-fermentable reducing substance in mercury filtrates from 2 patients with advanced hypertensive disease.

This report describes primarily the determination of non-fermentable reducing substance and non-urea nitrogen in protein-free blood filtrates from hypertensive subjects. The patients are classified into 2 groups, one with normal, the other with elevated blood non-protein nitrogen levels, in an effort to demonstrate any corresponding variations in the fractions under consideration.

EXPERIMENTAL PROCEDURE

Blood for analysis was drawn into flasks containing sodium oxalate as anticoagulant. Deproteinization and analysis were performed promptly. Some filtrates were stored overnight in stoppered bottles, in a cold room at 3 to 4° C., pending analysis. This did not appear to affect the results in any way. Most of the blood samples were obtained under fasting conditions. Urea clearances were determined by a method described in 1928 (4). Mean blood pressures were recorded.

ANALYTICAL METHODS

Zinc hydroxide precipitation was performed according to Somogyi (2). Tungstic acid precipitation was carried out by the Folin and Wu (5) technic, as modified by Haden (6). These methods were selected because of their convenience for the study of reducing and nitrogenous fractions. Tungstic acid filtrates contain apparently maximum amounts of these fractions, which are considerably restricted in zinc filtrates, by removal of glutathione, ergothionine, uric acid, etc. Non-protein nitrogen was determined by the method of Folin and Wu (5), by use of superoxol to clear the digestion mixtures when necessary. Urea nitrogen was estimated by the gasometric hypobromite method of Van Slyke and Kugel (7). Total reducing substance was determined with the copper-iodometric reagent of Shaffer and Somogyi (8), containing 5 grams of potassium iodide per liter. For non-fermentable reducing substance, a reagent containing 1 gram of potassium iodide per liter was employed. Glucose was removed with washed yeast (Fleischmann's starch-free baker's yeast) by the method of Somogyi (2). All analyses were made in duplicate.

Values for non-fermentable reducing substance have been given in terms of glucose-equivalents, and therefore have only relative significance. The limits of error in the analytical method employed for estimating this fraction were found to be less than 1 mgm. of glucose per 100 cc.

RESULTS

Table I presents the results on analysis of zinc filtrates from the control subjects. The ranges of non-protein nitrogen and urea are within the limits given by Somogyi (2) and by Peters and Van Slyke (9). The analytical results for non-fermentable reducing substance are in fair agreement with those given by Somogyi (2), there being no appreciable amount in 17 out of 21 filtrates.

Table II contains the findings on analysis of zinc filtrates from a group of hypertensive subjects with blood non-protein nitrogen below 30 mgm. per 100 cc. The range of the nitrogen fractions is very close to that in Table I. Appreciable non-fermentable reducing substance is present in 20 out of 27 filtrates. In 13 instances, the amount is greater than that found in any control filtrate.

Table III gives analytical data on zinc filtrates from a group of hypertensive subjects with blood

TABLE I

Observations on zinc filtrates from control subjects

Subject	Age	Diagnosis	Blood pressure	Urea clearance	N P N	Urea N	Non-urea N P N	Reducing substances, estimated as glucose	
								Total	Non-fermentable
	years			*per cent of normal*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
1	21	Acute lead poisoning, latent syphilis, secondary anemia	124/80		24	10	14	92	0
2	35	Diabetes mellitus, 40 units insulin daily	115/80	95	22	13	9	84	0
3	18	Subacute appendicitis	112/64	118	19	9	10	68	0
4	26	Rheumatic heart disease	140/20	73	33	19	14	75	0
5	40	Bronchial asthma	124/100	127	25	17	8	58	1
6	50	Diabetes mellitus	126/86	81	21	13	8	222	0
7	34	Acute salpingitis	118/70		18	7	11	62	0
8	19	Gigantism, traumatic ulcer	140/80	108	20	11	9	86	2
9	24	Healthy	108/64	121	17	8	9	74	0
10	17	Allergic eczema	124/88	109	19	12	7	50	0
11	27	Healthy	112/70	88	21	10	11	78	0
12	18	Peptic ulcer	122/82	111	19	10	9	68	0
13	18	Sacro-iliac strain	120/75	132	23	12	11	75	1
14	41	Chronic sinusitis, chronic cholecystitis	124/80		26	17	9	60	0
15	59	Allergic dermatitis	114/72	82	19	8	11	73	0
16	37	Healthy	125/75	83	22	11	11	78	0
17	23	Bronchial asthma	95/70	99	20	7	13	45	0
18	23	Healthy	112/70	116	20	12	8	88	0
19	24	Acute tonsillitis, rheumatic arthritis	130/86		23	11	12	98	0
20	22	Healthy	138/84	95	18	8	10	82	0
21	27	Gonorrheal arthritis	150/88	110	23	12	11	64	1
Mean	29		123/75	103	22	11	10	80	0.2
Standard deviation				±16.6	±3.6	±3.2	±1.9	±34.3	±0.5

non-protein nitrogen of 30 mgm. per 100 cc. and over. Corresponding to expectation, there is elevation of the non-urea nitrogen as compared with Tables I and II. Demonstrable amounts of non-fermentable reducing substance are present in 10 out of 11 filtrates, with a mean value more than double that in Table II. The difference between these means appears significant, since its probable error is ± 0.5 mgm. per 100 cc., or less than one-fourth of the difference.

There does not appear to be any correlation between the amounts of non-fermentable reducing substances shown in Tables II and III with blood pressures. The relation to urea clearances is uncertain. In Table III, the two highest values for non-fermentable reducing substance occur in subjects with clearances below 10 per cent of normal. With higher clearances, there does not appear to be any correlation. There is some tendency for non-urea nitrogen to vary with non-fermentable reducing substance, the correlation coefficients in Tables II and III being +0.26 and +0.30, respectively.

Classification of the subjects in Tables II and III according to clinical diagnoses shows that appreciable amounts of non-fermentable reducing substance were present in each of 6 cases of malignant hypertension, with a mean value of 3.3 mgm. per 100 cc. (referred to glucose); in 15 out of 21 cases of benign hypertension, with a mean value of 1.9 mgm. per 100 cc.; and in 4 out of 5 cases of arteriosclerosis with hypertension, with a mean value of 2.0 mgm. per 100 cc.

Tungstic acid filtrates from groups of control and hypertensive subjects without nitrogen retention were analyzed in a similar way. The mean values for non-protein nitrogen in the two groups were almost identical: 24.9 mgm. per 100 cc. for the controls, 24.8 mgm. per 100 cc. for the hypertensives. The mean value for non-fermentable reducing substance in the control group was 20.9 mgm. per 100 cc., in close agreement with

the figure of 21 mgm. per 100 cc. given by Somogyi (2). A mean value of 22.3 mgm. per 100 cc. was obtained in the hypertensive group. The variation within the groups was such as to tend to obscure the significance of any difference between the means.

A few preliminary experiments were made relative to the non-fermentable reducing substance present in zinc filtrates from subjects with hypertension. It appeared to be stable on storage in the cold and to reside chiefly in the plasma. Attempts at acid hydrolysis of filtrates resulted in little or no change in the amount of non-fermentable reducing substance.

DISCUSSION

As suggested by Hiller, Linder, and Van Slyke (1), it is entirely possible that retained nitrogenous substances, such as uric acid and creatinine, may account for abnormal reducing properties in appropriate blood filtrates. Somogyi (2) found that zinc filtrates of blood from normal subjects prepared by his method contain somewhat less creatinine than tungstic acid filtrates and are free from uric acid, glutathione, and ergothionine. The nature of the non-urea nitrogen and non-fermentable reducing substance in these filtrates remains largely unknown. Retention of crea-

TABLE II

Observations on zinc filtrates from hypertensive subjects with non-protein nitrogen below 30 mgm. per 100 cc.

Subject	Age	Diagnosis	Blood pressure	Urea clearance	N P N	Urea N	Non-urea N P N	Reducing substances, estimated as glucose	
								Total	Non-fermentable
	years			*per cent of normal*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
22	33	Essential hypertension, hyp. ht. dis., chr. bronchitis	190/110	80	21	8	13	80	5
23	44	Essential hypertension, hyp. ht. dis.	155/105	85	26	17	9	84	1
24	50	Essential hypertension, hyp. ht. dis.	268/140	79	18	10	8	178	0
25	54	Essential hypertension, hyp. encephalopathy	190/108	103	22	12	10	79	4
26	47	Essential hypertension, hyp. ht. dis.	178/98	79	24	12	12	78	0
27	21	Malignant hypertension	220/118	84	21	12	9	148	2
28	54	Essential hypertension	168/98	92	20	12	8	143	1
29	37	Essential hypertension, hyp. ht. dis., thyroidectomy	218/110	77	21	11	10	54	1
30	52	Essential hypertension	190/130	126	26	14	12	99	2
31	33	Malignant hypertension, lt. lumbar sympathectomy	174/110	97	17	8	9	87	4
32	39	Essential hypertension, hyp. ht. dis.	220/100		17	10	7	150	1
33	54	Arteriosclerosis with hypertension	264/130	96	20	8	12	43	3
34	66	Essential hypertension, hyp. ht. dis.	185/90	171	17	8	9	82	3
35	35	Malignant hypertension, CNS syphilis	220/130	90	23	14	9	106	1
36	46	Arteriosclerosis with hypertension	176/110	80	25	13	12	62	1
37	35	Essential hypertension, hyp. ht. dis.	176/100	85	18	7	11	89	0
38	49	Essential hypertension, diabetes, obesity, goiter	204/114	87	19	9	10	57	0
39	53	Essential hypertension	210/115	157	19	8	11	132	3
40	39	Essential hypertension	196/128	83	19	10	9	94	0
41	37	Thyrotoxicosis, thyrotoxic ht. dis.	181/85	133	20	8	12	72	3
42	28	Malignant hypertension, hyp. encephalopathy	180/130	55	29	18	11	63	3
43	58	Essential hypertension, hyp. ht. dis.	214/126	68	17	9	8	90	1
44	68	Arteriosclerosis with hypertension, hyp. ht. dis.	200/110	68	22	13	9	87	0
45	66	Essential hypertension, hyp. ht. dis.	185/90	59	20	10	10	64	3
46	56	Hyperthyroidism	200/104	45	24	16	8	112	2
47	62	Essential hypertension, hyp. ht. dis.	194/104	59	27	13	14	126	0
48	42	Essential hypertension	170/120	63	18	6	12	107	3
Mean	47		197/112	89	21	11	10	95	1.7
Standard deviation				±29.7	±3.4	±3.1	±1.8	±33.1	±1.5

TABLE III

Observations on zinc filtrates from hypertensive subjects with non-protein nitrogen 30 mgm. per 100 cc. and over

Subject	Age	Diagnosis	Blood pressure	Urea clearance	N P N	Urea N	Non-urea N P N	Reducing substances, estimated as glucose	
								Total	Non-fermentable
	years			*per cent of normal*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
49	59	Arteriosclerosis with hypertension	190/100	78	36	24	12	78	3
50	45	Essential hypertension	190/125	19	61	42	19	68	3
51	40	Malignant hypertension	212/142	8	84	62	22	66	7
52	57	Malignant hypertension, hyp. ht. dis.	260/160	48	42	31	11	76	3
53	67	Essential hypertension, hyp. ht. dis., cor. art. dis.	218/110	43	36	21	15	100	4
54	62	Essential hypertension, cor. art. dis.	220/98	59	30	22	8	101	5
55	24	Chronic hemorrhagic nephritis	155/95	32	38	23	15	82	0
56	58	Arteriosclerotic nephritis	180/120	24	45	35	10	94	3
57	59	Arteriosclerosis with hypertension	220/148	15	85	72	13	164	3
58	28	Chronic hemorrhagic nephritis, uremia	220/115	4	135	118	17	73	8
59	29	Pituitary basophilism?	180/110	58	31	21	10	151	4
Mean	48		204/120	35	57	43	14	96	3.9
Standard deviation				±23.8	±32.6	±30.4	±4.3	±32.9	±2.2

tinine might account for the larger amounts found in Table III, as compared with Table II. A slight association between these fractions is suggested by analysis of the data. The correlation is of a low order, however, and the tendency for non-fermentable reducing substance to appear before the non-protein nitrogen rises suggests that the former may contain little or no nitrogen under the conditions imposed. This would be in agreement with the observations of West, Scharles, and Peterson (3) on mercury filtrates, which were found to contain increased amounts of non-fermentable reducing substance in 2 cases of hypertensive disease, but were generally free from nitrogen.

It may be significant that the two highest values for non-fermentable reducing substance in zinc filtrates occurred in those from patients with urea clearances less than 10 per cent of normal. If the limited number of subjects and the variation inherent in the urea clearance are taken into account, the apparent absence of progressive accumulation of this reducing fraction with moderate decrease in renal function suggests that it is either readily excreted or destroyed under such conditions. The increase with severe renal damage might be interpreted to mean that it is eliminated largely via the kidneys,—perhaps by a mechanism analogous to that for urea, which does not tend to accumulate in the body fluids until its renal clearance has been lowered to less than half its usual value. This would be in agreement with the observation by West (10) that appreciable amounts of non-fermentable reducing substances are present in normal urine. West pointed out that the amount eliminated is related to the carbohydrate intake, and particularly to the ingestion of caramelized material. Whether this has any bearing on the non-fermentable reducing substance noted in the present study is a matter for investigation.

Attention is called to the possibility that reducing substances might tend to accumulate in the blood or urine by interference with renal oxidations. Glaser, Laszlo, and Schürmeyer (11) have shown that mammalian kidneys normally require up to 20 per cent of the total oxygen uptake. These investigators, confirmed by Van Slyke, Rhoads, Hiller, and Alving (12), report a linear relationship between renal blood flow and oxygen consumption, both of which are unrelated to excretory activity. Studies of hypertensive subjects by indirect methods, summarized recently by Foà, Woods, Peet, and Foà (13), indicate a fairly general tendency toward lowering of renal blood flow in these subjects. It would seem to follow, *pari passu,* that there might exist a corresponding tendency toward impairment of

renal oxygen consumption. As a working hypothesis, it is suggested that renal ischemia, by interfering with intense cellular oxidations, may give origin to unusual amounts of incompletely oxidized substances. This hypothesis should be susceptible to direct experimental examination.

The preliminary examination of zinc filtrates containing non-fermentable reducing substance suggests that it is stable and exists chiefly in the plasma. This latter finding is in contrast with the studies of Somogyi (2) on tungstic acid filtrates, in which the bulk of the non-fermentable reducing fraction was shown to originate in the cells.

SUMMARY

1. Protein-free blood filtrates from subjects with and without arterial hypertension have been examined for non-fermentable reducing substance and non-urea nitrogen content.

2. Appreciable amounts of non-fermentable reducing substance were noted in 30 out of 38 zinc filtrates from patients with hypertensive disease, and in 4 out of 21 control filtrates. A similar difference was found in tungstic acid filtrates.

3. Among the hypertensive subjects, the non-fermentable reducing substance in zinc filtrates exhibited some tendency to vary with non-urea nitrogen. No direct relation to blood pressure or urea clearance could be demonstrated.

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