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Effect of Acetylsalicylic Acid Ingestion on Electrophoretic Patterns of Human Plasma.*

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In their work with the anti-clotting agent dicoumarol, Link *et al.*[1] presented *in vitro* evidence that salicylic acid was a degradation product of the widely used antipyretic and analgesic acetylsalicylic acid. Following the work of Link, Field[2] discovered that lactating rats, which normally have high levels of fibrinogen and prothrombin, suffered from hypoprothrombinemia after ingestion of dicoumarol; if dicoumarol was replaced by acetylsalicylic acid a much less severe hypoprothrombinemia resulted. Rapoport and Guest[3] found a decreased sedimentation rate of erythrocytes in subjects receiving salicylic acid or its acetyl derivative, but admitted that their results may have been influenced by certain uncontrolled factors. They contended that the chief factor in regulation of sedimentation rates was plasma fibrinogen. This statement was confirmed by Allen *et al.*;[4] however, these investigators found no hepatic pathology in dogs fed fatal doses of dicoumarol. It was claimed by Homburger[5] that salicylates produce a fall of plasma fibrinogen proportional to total dose rather than to blood levels. His results are subject to question since they are based on a study of only 3 patients, and these had metastatic carcinomas. It has also been claimed by Shapiro, Redish, and Campbell[6] that the ingestion of acetylsalicylic acid increases the clotting time of the blood.

All of the work cited above was based either on the method of Quick[7] or of Cullen and Van Slyke.[8] There are definite theoretical disadvantages in both of these. Quick's method measures clotting time, and depends on the addition of calcium and thromboplastin to a diluted plasma. The dilution is unphysiological and is also subject to salt errors.[9] In Cullen's method a fibrin precipitate is weighed and analyzed for nitrogen; the results are expressed as either prothrombin or fibrinogen. Since the stoichiometry of fibrin formation *in situ* is not known, the method is at best somewhat equivocal.

It appeared to us that electrophoresis might be applied advantageously to this problem, since it is capable of separating the plasma proteins with a minimum of denaturation and loss. While prothrombin is not ordinarily present in sufficient concentration to appear on an electrophoretic pattern, fibrinogen is a well defined component of such patterns.

Materials and Methods. Samples of oxalated blood were obtained from normal healthy male and female subjects before and after a 7-day period during which they ingested 4 g of acetylsalicylic acid per day. The plasma was diluted with 3 volumes of a barbiturate buffer (pH = 8.63, ionic strength—0.1, sodium diethyl barbiturate—0.1N), and dialyzed at 2°-6°C for 3 days with daily change of buffer. Dialysis was performed in Visking casings. The final volume of buffer

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2 Field, J. B., *Am. J. Physiol.*, 1945, **143**, 238.

3 Rapoport, S., and Guest, G. M., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 43.

4 Allen, E. V., Barker, N. W., and Waugh, J. M., *J. Am. Med. Soc.*, 1942, **120**, 1009.

5 Homburger, F., *Am. J. Med. Sciences*, 1946, **211**, 346.

6 Shapiro, S., Redish, M. H., and Campbell, H. A., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 12.

7 Quick, A. J., *J. Am. Med. Soc.*, 1938, **110**, 1658.

8 Cullen, G. E., and Van Slyke, D. D., *J. Biol. Chem.*, 1920, **41**, 587.

9 Deutsch, H. F., and Gerarde, H. W., *J. Biol. Chem.*, 1946, **166**, 381.

TABLE I.
Electrophoretic Analysis of Plasma Proteins.

Case No.	Normals						Experimentals					
	Alb.	Globulins					Alb.	Globulins				
	A	α_1	α_2	β	Φ	γ	A	α_1	α_2	β	Φ	γ
1	58.5	5.71	7.38	10.71	5.98	11.83	57.3	6.42	7.74	11.84	5.76	11.00
2	56.6	5.80	9.20	11.10	5.50	11.90	57.5	4.10	9.47	13.38	6.31	9.28
3	57.2	2.96	9.98	11.60	8.90	9.70	53.5	3.00	10.70	14.40	8.75	9.70
4	66.8	4.46	8.00	11.24	3.42	6.09	66.9	6.37	7.33	10.85	3.82	4.77
5	57.8	5.41	10.80	11.94	5.40	8.80	57.8	4.53	11.83	14.04	4.76	7.48
6	67.8	4.24	8.47	9.54	3.18	6.71	64.2	6.60	8.80	9.74	4.07	6.61
7	55.9	4.86	10.77	12.15	6.25	10.08	56.0	5.73	7.29	12.50	6.25	11.97
8	64.6	4.98	6.64	11.00	4.67	8.15	61.0	6.67	9.60	11.92	4.36	6.38
9	61.1	5.85	11.99	8.77	6.43	5.85	62.7	4.94	10.64	7.99	5.70	7.98
10	61.1	3.97	10.11	11.55	4.33	9.04	63.7	4.24	8.50	11.89	3.68	7.92
11	61.7	3.40	7.71	9.25	5.24	12.67	61.4	4.78	7.97	9.56	4.78	11.56
12	58.7	3.30	10.19	12.28	5.09	10.48	55.9	4.54	9.41	11.37	7.79	11.04
13	68.3	5.40	7.04	11.22	3.85	4.16	66.6	7.47	7.78	9.98	3.74	4.36
14	58.3	4.39	9.86	11.23	7.12	9.04	56.0	5.06	9.11	12.16	7.60	10.13
15	67.7	5.32	9.88	9.51	2.66	4.94	67.7	4.30	9.41	8.06	2.42	8.05
16	62.3	5.30	13.26	9.47	5.69	6.06	61.2	6.08	11.02	11.79	5.30	4.56
Avg	61.5	4.70	9.45	10.78	5.23	8.46	60.6	5.30	9.13	11.34	5.32	8.29
Std. Dev.	4.17	0.90	1.70	1.08	1.52	2.51	4.31	1.07	1.32	1.90	1.68	2.39

Std. Dev. of Changes*	A	α_1	α_2	β	Φ	γ
	1.45	0.83	1.26	0.97	0.76	1.09

* Calculated for each component based on difference between normal and experimental values.

was sufficient to fill the apparatus completely. Electrophoresis was carried out in the Longsworth[10] modification of the Tiselius[11] apparatus. The patterns were photographed and projections (2.5X) were traced on bond paper. The area enclosed by the base line and the protein peaks was measured with a planimeter and equated to total protein. Each peak was measured separately to determine the individual proteins. Mobility measurements were made from the boundary anomaly of the pattern. While both ascending and descending boundaries were compared and analyzed, only data from the descending boundaries is included.[10] Changes in total protein content were checked refractometrically, and standardized against a sample of crystalline serum albumin dissolved in the buffer. No set of samples (normal and experimental) showed a greater than 0.5% variation in protein content by this method.

Conditions of the electrophoretic experiments were carefully controlled. The current was held at a value of 25 $\pm$ 0.5 milliamperes, and was applied for 120 $\pm$ 1 minutes. The maximum variation of refractive increment was $\pm$ 0.0003. The conductivity of each solution was also measured, and the greatest difference observed in any pair of samples was $\pm$ 0.0004 ohms^{-1}. In measurements of area by the planimeter, the sum of the areas or the peaks due to protein was 100 $\pm$ 0.1%. This agreed with the measurement of the total area within the same limits.

Results. The detailed analysis of each experiment is presented in Table I. Average values agree well with those of other workers,[12,13] and the standard deviations are also in fairly good agreement. Differences between our results and those of others is largely due to the fact that we used the Pedersen[14] method of area division while others have used the method of Tiselius and Kabat.[15] The

[10] Longsworth, L. G., *Chem. Rev.*, 1942, **30**, 323.

[11] Tiselius, A., *Trans. Faraday Soc.*, 1937, **33**, 524.

[12] Dole, V. P., *J. Clin. Invest.*, 1944, **28**, 708.

[13] Bieler, M. M., Ecker, E. E., and Spies, T. D., *J. Lab. Clin. Med.*, 1947, **32**, 132.

[14] Svedberg, T., and Pedersen, K. O., *The Ultracentrifuge*, p. 296, Oxford University Press, London, 1940.

[15] Tiselius, A., and Kabat, E. A., *J. Exp. Med.*, 1939, **69**, 119.

latter of these methods introduces errors discussed by Svensson.[16]

Discussion. Our results indicate that for the 16 cases studied, none of the protein components accessible to electrophoresis shows any marked change. Particularly, only 4 of the 16 cases showed a loss of 0.5% in fibrinogen, while as many cases showed a gain of the same magnitude. Witts[17] has published the statement that fibrinogen levels may be reduced 30% before prothrombin time is significantly prolonged. With respect to the earlier claims cited above, our results indicate that the change in clotting time and sedimentation rates following acetylsalicylate ingestion is not due to marked changes in fibrinogen levels. Actually, the presence of any large asymmetric molecule can change sedimentation rate regardless of protein concentration.[18,19] Whether this is due to an effect on protein surface charge or to chemical interaction is not known. Smith[20] claims that salicylates are bound to some plasma proteins.

Ham and Curtis[21] have reported a very careful study of the relation between hepatic function and plasma proteins. These authors concluded that plasma fibrinogen was an especially sensitive index of liver disease or dysfunction. They stated that in damaged livers the plasma fibrinogen was sharply decreased. If these reports are correct then our results indicate that salicylates are not hepatotoxic, and likewise, any effect of salicylates on prothrombin time is not reflected by corresponding changes in fibrinogen levels.

Summary. 1. Blood of normal subjects was examined by electrophoresis before and after ingestion of 4 g of acetylsalicylic acid per day for 7 days. Physical and chemical constants obtained were in good agreement with published values.

2. No marked changes were observed in any protein components accessible to electrophoresis. The conclusion is drawn that the effect of salicylates on blood clotting time and sedimentation rates of erythrocytes cannot be due to a reduction of plasma fibrinogen, and that salicylates are probably not hepatotoxic.

16 Svensson, H., *Ark. Min. Kemi och Geol.*, 1946, **22**A, 1.

17 Witts, L. J., *J. Path and Bact.*, 1942, **54**, 516.

18 Zozaya, J., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 182.

19 Yardumian, K., *Am. J. Clin. Path.*, 1937, **7**, 105.

20 Smith, P. K., Gleason, H. L., Stoll, C. G., and Ogorzalek, S., *J. Pharm. and Exp. Therap.*, **1946, 87**, 237.

21 Ham, T. H., and Curtis, F. C., *Medicine*, 1938, **17**, 413, 447.

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Development of Renal Function in Fetal and Neo-Natal Rabbits Using Phenolsulfonphthalein.*

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At birth mammals undergo tremendous physiological adjustments in their respiration, nutrition, circulation and excretion correlated with the achievement of independence of the material circulation, Much attention has been directed toward the first 3 of the above mentioned adjustments but fewer physiological studies have been made on the development of an independent renal excretion. Most of the work reported indicates that there is some formation of urine in the terminal stages of uterine life.[1,2,3] It has been the purpose of our investigations to try to determine to

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