Studies on Salicylates and Complement in Diabetes

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SUMMARY

Increased levels of complement have been found in diabetic patients, being often more marked in young patients with proliferative retinopathy. Neither duration of diabetes nor the giving of insulin was associated with any particular alteration of complement levels. Acetylsalicylic acid was effective in suppressing complement in vitro in all instances, but to a greater extent in the younger, more severely affected patients.

These findings are discussed and related to mechanisms in production of retinopathy. DIABETES *15*:730-33, October, 1966.

The low incidence of retinopathy in patients who have both diabetes and rheumatoid arthritis, and who are taking large doses of salicylates, has been recently reported.[1,2] It had been found, however, that in a series of diabetic patients with progressive retinopathy, even large doses of salicylates cannot halt or reverse changes already present in the eye. These findings have led to an investigation of the possible roles of salicylates and complement in this situation. Quantitative estimations have been made of complement levels in diabetes and diabetic retinopathy and of the effects of acetylsalicylic acid in vitro on these levels.

METHODS

Sera were obtained from three groups:

Group A: Twelve volunteers aged twenty-two to forty-two years (mean, thirty years).

Group B: Fifteen diabetic patients attending the Out-Patient Clinics. Some had occasional, transient appearance of microaneurysms, single exudates, or small hemorrhages. Their ages ranged from eighteen to fifty-six years (mean, 40.3 years); duration of diabetes zero to twenty-three years (mean, 7.3 years). Age at diagnosis ranged from one to fifty-six years (mean, thirty-three years).

Group C: Fifteen diabetic patients with proliferative retinopathy, under assessment for pituitary ablation. Their ages ranged from twenty-one to fifty-three years (mean, thirty-two years); duration of diabetes seven to twenty-seven years (mean, 18.7 years). Age at diagnosis ranged from three to thirty years (mean, 12.5 years).

Blood samples were collected and serum separated at 4° C., before storage at −40° C.

Complement titration was carried out by the method of Kabat and Mayer[3] and expressed in C'50 units. Duplicate series containing identical dilutions of serum diluted 1/25-1/100 were set up. One series of each serum contained 0.5 cc. buffer, the other 0.4 mg. acetylsalicylic acid in 0.5 cc. buffer adjusted to *p*H 7.4 (final dilution 33 mg./100 ml.). With the addition of 0.2 ml. sensitized sheep cells, the final volume was made up to 1.2 ml. After thorough mixing and incubation at 37° for thirty minutes, tubes were centrifuged, and degree of hemolysis read in a Coleman colorimeter at 541 mμ, the value of C'50 units calculated from a log graph.

RESULTS

Effects of presence of diabetes and retinopathy.

Complement levels in each group are shown on figure 1, and the values for the diabetic patients tabulated on table 1. The mean value of the normal Group A is 36.8 U. (S.D., 5.6 U.); of Group B (diabetic), 48.5 U. (S.D., 7 U.); and of Group C (diabetic proliferative retinopathy), 53.1 U. (S.D., 10 U.). The combined levels of both diabetic groups are significantly higher than the normal population ($P = 0.05$), but the difference between the two diabetic groups taken as a whole, with and without severe progressive retinopathy, is not statistically significant. Those under thirty years of age in Group C had higher complement levels (mean, 56.7 U.—S.D., 12.9) than the older patients (mean, 49.3 U.—S.D., 6.9) in the same group. The older patients in this group showed levels agreeing closely with the over-all average of Group B (mean, 48.5 U.—S.D.,

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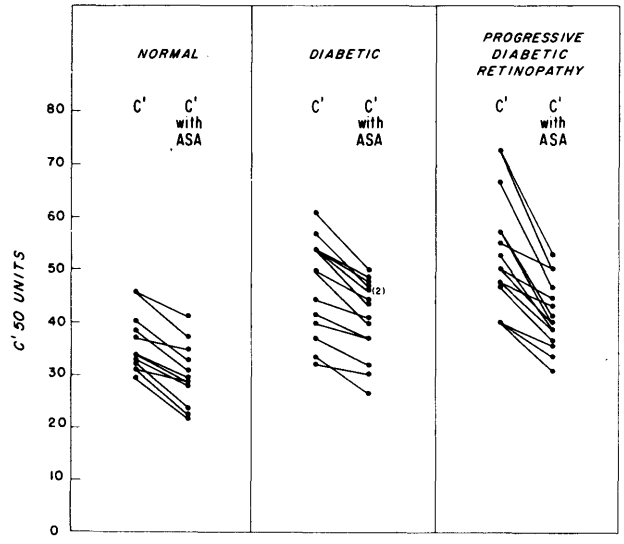

FIGURE 1

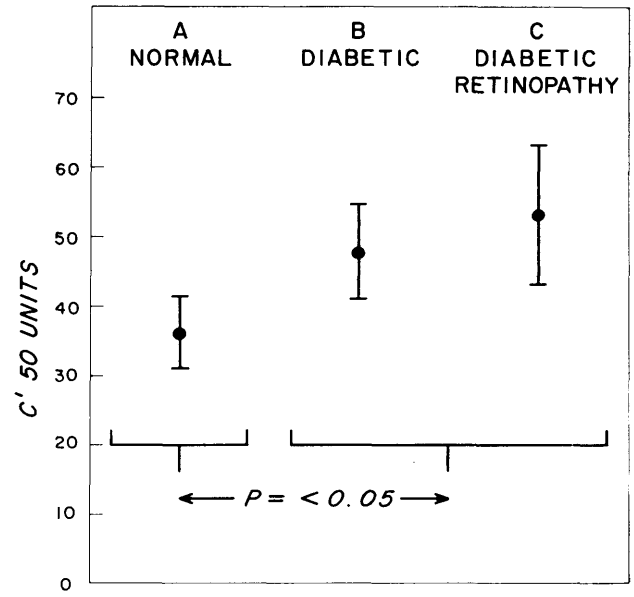

FIGURE 2

7). The variation in complement values in Group B occurred throughout the age range. In the normal group, where the range was narrower, no association of complement level with age could be found.

No trend in complement levels could be associated with either duration or age of onset of diabetes. The three newly discovered diabetics of Group B had as high levels as any in the group. Similarly, the giving of insulin has not been associated with especially high or low levels of complement.

Effects of salicylate on complement in vitro: (figure 2)

An inhibition of complement by salicylates in vitro was found in every instance. A similar degree of inhibition was found in the normal (15 per cent) and in the diabetic Group B (14 per cent). The group with severe retinopathy showed a greater mean inhibition: 20 per cent ($0.10 < P < 0.05$), most marked in those under thirty years of age (22 per cent).

TABLE 1

Complement levels in diabetes

Group B						Group C				
Sex	Age	Age at diagnosis	Insulin	Mild ret.	C'50	Sex	Age	Age at diagnosis	Insulin	C'50
M	18	1	50	0	50	M	21	9	65	50
F	22	8	56	+	42	F	22	9	38	57
M	25	20	28	0	50	M	23	5	80	71
M	28	12	39	+	57	F	26	4	38	57
M	38	23	52	+	37	F	27	3	38	71
M	39	27	48	0	32	F	28	9	34	66
F	41	33	28	+	40	M	28	14	40	40
M	43	38	—*	+	45	M	28	11	56	40
M	44	44	—†	0	61	M	31	13	48	53
M	47	45	15	0	54	F	35	17	30	47
M	48	41	54	0	54	M	36	15	45	40
M	50	45	—*	0	54	M	36	15	—†	55
M	50	50	—†	0	44	M	40	13	40	47
M	56	56	—*	0	54	M	47	20	40	55
F	56	53	—†	0	54	F	53	30	28	47
Mean =	40.3	33			48.5	Mean =	32	12.5		53.1

*Has received Regular Insulin for past acute illnesses
†Has never received insulin

DISCUSSION

Increase in complement above normal levels must signify either an increase in synthesis or reduction in decay or consumption.

Fischel[4] has found acutely increased levels of complement in many unrelated conditions such as tonsillitis, myocardial infarction, thyrotoxicosis, and drug allergies; and it is evident that serum complement can increase in response to a variety of nonspecific stimuli. Balch[5] has also found increased levels of complement in diabetes, but could not correlate the levels with the presence or absence of peripheral vascular disease. Páv[6] has found that up to 33 per cent of a group of diabetics who had never received insulin exhibited complement consumption by insulin in vitro. This proportion was even higher in those treated with insulin, but was not present in all diabetics tested. A small proportion of normal people (4 per cent) also showed consumption of their complement by insulin. Chetty et al.[7] have repeated this work and get greater proportions of all groups capable of this reaction with insulin. Although Páv suggests this may be due to more than one type of antibody, the possibility arises that at least some of this complement consumption may be associated with the proteolytic action of insulin.[8] Our results do not suggest any association of complement with the giving or withholding of insulin to our patients. Complement has been demonstrated in the glomeruli in diabetic glomerulonephritis,[9,10] and Burkholder[11] has recently demonstrated the avidity of these lesions for heterologous complement. The rapid fixation of complement in the tissues in disseminated lupus erythematosis and acute membranous nephritis[12] has been held responsible for the low levels of complement in these conditions; and the finding of high levels in diabetes, along with these reports of complement consumption and fixation in vivo and in vitro, becomes, therefore, all the more significant.

Marucci[13] has reported the effects of age on complement levels; he found a small (3 per cent), but significant rise in complement with age, detectable at each ten-year interval between twenty-one and fifty years, or 10 per cent over-all. Our groups fall within this age span. The mean age of diabetic Group B is ten years greater than that in Group A, and therefore, a 3 per cent rise in complement could be expected; however, the average increase in complement is 24 per cent. Group C (diabetic proliferative retinopathy) has a mean age similar to Group A, but here there is a 44 per cent average increase in complement. Variation with age was not apparent either in normal Group A or in Diabetic Group B. The patients in Group C, with severe retinopathy, appeared to have complement levels in inverse ratio to their age, as the three highest levels were seen in patients under thirty years of age. It has been our experience that patients under thirty years often show a more malignant tempo of deterioration in their eye disease than older patients.

Complement levels were found to be as high in newly discovered diabetics as in those who had diabetes for ten years or more. The increase noted in our patients, none of whom had any acute lesion which might raise complement titers temporarily, suggests a continuing focus of injury, present not only when complications are progressive, but also at a time when they cannot yet be detected clinically, as in about 2/3 of our Group B. This indication of early and continuing pathology is in agreement with the finding of retinal extravascular fluorescence and microaneurysms detected by dye injection studies before they can be detected by ordinary clinical tests,[14] and the presence of basement membrane and elastic tissue changes which may predate the diagnosis of chemical diabetes.[15] Other evidence that injury is occurring in diabetes is gained from the descriptions of endothelial proliferation by Blumenthal et al.,[16] and of arteriovenous shunting by Cogan et al.[17] These changes, together with the increase in vascular permeability referred to, have been described by Kulka[18] in endogenous reaction to nonspecific injury.

Van Oss[19] has shown an in vitro inhibition of complement by acetylsalicylic acid. This finding has been criticized by Anderson[20] who thought the suppression had been caused by nonspecific alteration in *p*H of the incubating medium. In our study, however, inhibition by acetylsalicylic acid was found to be maximal at *p*H 7.4, and was less effective or absent at a lower *p*H. Mills and Levine[21] have investigated the complement-inhibitory action of salicylaldoxine and related compounds, including methyl salicylate and salicylamide. The former compound produced 50 per cent inhibition at a concentration of 5.0×10^{-4} M, and the latter two at 33×10^{-4} M. The concentration of 33 mg. per 100 ml. acetylsalicylic acid used in the present study corresponds to 18.3×10^{-4} M, at which level an average 16 per cent inhibition of hemolysis was detected. We are in agreement with van Oss and Mills and Levine that sodium salicylate does not inhibit complement in vitro. Mills and Levine suggest that the inhibition by salicylaldoxine is due to prevention of effective combination of C'_3 with EAC_{142}. As the suppression is somewhat

greater in our younger diabetics with proliferative retinopathy, the rise in complement level in this group may be partly due to components involved in that reaction; but alterations in the other components or interactions must also be responsible.

These observations can be interpreted as evidence of chronic continuing injury with overproduction of complement and provoke further consideration of autoimmune mechanisms in the pathogenesis of diabetic retinopathy. The interference with complement action by salicylates demonstrated here may be related to the apparent prevention of retinopathy in diabetic patients treated with these drugs over prolonged periods. However, changes already present are unlikely to be affected.

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