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THE PROTECTIVE EFFECT OF SODIUM SALICYLATE ON SERUM INDUCED MYOCARDITIS IN RABBITS

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With the description by Rich and Gregory (1943) of a method by which lesions resembling those found in human rheumatic fever could be produced in rabbits, it has been possible to investigate the effects of various therapeutic agents on the development of these lesions, particularly those in the heart. We recently reported (Macgregor and Wood, 1949) that the histamine antagonists, benadryl and neoantergan, did not prevent the development of myocardial lesions in serum sensitized animals. Other reports have since confirmed these results, which were contrary to the earlier findings of Kyser, McCarter, and Stengle (1947).

In view of the known lack of action of these substances in human serum sickness, and the fact that sodium salicylate has a favourable effect in this disease, it was decided to test whether under our conditions sodium salicylate would influence the myocardial lesions produced in rabbits. Not only would this act as a positive control for our negative results with neoantergan and benadryl, but it would also be of interest in view of the use of sodium salicylate in the treatment of rheumatic fever.

Interest in the possible curative action as opposed to symptomatic benefit of salicylates in the treatment of human rheumatic fever has been stimulated by the recent reports of Coburn and Moore (1942) and Coburn (1943), who have shown that if an adequate concentration of salicylate is maintained in the plasma, there is a much reduced incidence of valvular lesions in the hearts of patients with rheumatic fever. Coburn suggests that a plasma salicylate concentration of at least 35 mg. per 100 ml. may be required " to suppress the rheumatic reaction." Lower concentrations may mask a " progressive inflammatory reaction." Reid (1948) has shown that if the criterion of cure is taken as the return to normal of the erythrocyte sedimentation rate, adequate oral administration of sodium salicylate can really cure the disease in addition to relieving the symptoms.

Some authors have been unable to show a protective effect of salicylate in the experimental animal (Thomas and Stringfield, 1945), but quite recently Smull,

Wissler, and Watson (1948) observed a reduction in the severity of lesions in salicylate treated rabbits, while Sullivan, Parker, and Hibbert (1948), starting salicylate treatment before any horse serum was injected, were able to prevent the development of arterial lesions. In their full report on experimental serum disease, Ehrich, Seifter, and Forman (1949) include results on six rabbits treated with salicylate in which the incidence of myocardial lesions was zero, compared with 100 per cent incidence in eight animals receiving the same batch of serum but no salicylate.

In this paper we give an account of our results obtained with sodium salicylate.

METHODS

Experimental serum disease was produced in rabbits, as described by Rich and Gregory (1943) except that we omitted the injection of 2 ml. horse serum two days before the second injection of 10 ml. serum per kg. Sterile horse serum was injected into twenty-four young rabbits (1.5–2.0 kg.) on the first and twentieth days, each animal receiving 10 ml. per kg. intravenously on each occasion. Two batches of serum were used, six controls and six treated animals being injected with serum from the same batch. A total of twelve rabbits were given sodium salicylate, beginning on the day of the first serum injection. In order to maintain the plasma concentration as far as possible throughout the 24 hours, two daily doses were given orally at 10 a.m. and 4 p.m., and a larger dose was injected subcutaneously at 10 p.m. In the first group of animals the doses given were 200 mg. per kg. orally, and 300 mg. per kg. subcutaneously, a daily total of 700 mg. per kg. Two of the animals died after six and eight days at this dosage level, and the amounts given were subsequently reduced to 150 mg. per kg. orally and 200 mg. per kg. subcutaneously for the remainder of the period. Another of the four surviving animals died on the eleventh day.

The second group of treated rabbits were given the smaller dosage throughout the experiment, two of them dying on the third and seventeenth days. Treatment was continued up to the twenty-sixth day, when the animals were killed and their hearts removed for section.

A few estimates were made of plasma salicylate concentration by means of the colorimetric method described by Brodie, Udenfriend, and Coburn (1944). The average concentration of salicylate in the plasma of six rabbits, about one hour after an oral dose of 150 mg. per kg. had been given, was 22 mg. per 100 ml. (range, 10 to 31 mg. per 100 ml.); at two hours two samples contained 28 and 18 mg. salicylate per 100 ml., and at $5\frac{1}{2}$ hours two samples contained 11 and 13 mg. per 100 ml. respectively.

RESULTS

Of the twelve treated animals, five died during the salicylate therapy on days 3, 6, 8, 11, and 17. Of the control group, two killed at 11 days and one dying accidentally at 17 days allow a comparison with treated animals dying at about the same time. It has been earlier shown by Ehrich *et al.* (1949) that subacute pathological changes can be produced by the first injection of serum alone, antibodies being present in the serum of the rabbit five to six days after a single injection of horse serum. In assessing the severity of the lesions produced in each animal we have again used the arbitrary system of awarding marks for the presence of intermuscular and perivascular infiltration, Aschoff-like bodies, valvular lesions, and for muscle and collagen

degeneration, as mentioned in our previous paper. The sections were examined by two observers independently, and in the second group of experiments (rabbits 920–931) it was not known until after the assessment had been made which sections were from treated and which from untreated animals. There were very obvious differences in the condition of the heart muscle, vessels, and valves, and except in rabbit 923 it was possible to predict whether the section was from a treated or untreated rabbit. From the few serum salicylate estimations made, there seemed to be no difference between the serum salicylate level in this animal and in the others which were protected. The results are shown in the Figure, where the height of each column represents the severity of the condition in one rabbit. The Figure emphasizes the difference between the control animals and those treated with sodium salicylate.

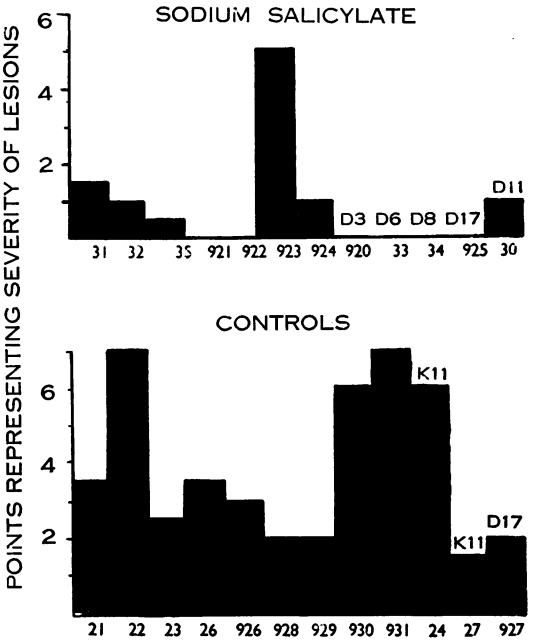

Figure showing the individual variation in the severity of the lesions produced in control and treated groups. Each column represents the severity of the lesions observed in one animal, points being allocated according to the severity of the observed lesions. (See text.) D3, K11, etc., indicate that the animal died on the 3rd or was killed on the 11th day. Where there is no superscription, the animal was killed on the 26th day. Figures under columns are individual animal numbers.

If only those animals which survived the full twenty-six days are included in the assessment it will still be seen that the lesions are less in the treated group. The nine control animals which survived were allocated a total of 36½ points, an average of about 4 points per animal. The total marks gained by the seven treated animals which survived until the 26th day were 9, an average of 1.3 per animal. It seems clear that the disease was very much less severe in animals treated with sodium salicylate.

Discussion

The mechanism by which salicylates inhibit the production of the pathological changes observed in these animals is still uncertain. Recent work has confirmed the *in vitro* activity of salicylates in inhibiting the activity of hyaluronidase which, it is suggested, plays a part in the breakdown of interfibrillar cement in rheumatic fever. It is interesting that sodium gentisate, a metabolic oxidation product of sodium salicylate, also inhibits hyaluronidase *in vitro* and has been found clinically useful in treating acute rheumatism. Earlier evidence on the effect of salicylates on the antigen-antibody reaction was inconclusive. It has been shown that the antibody titre does not seem to be correlated with the development of lesions. However, the work of Dammin and Bukantz (1949) suggests that there is a signi-

ficant correlation between the capacity to develop antibodies and the production of vascular lesions in rabbits as a result of serum injection. Sullivan *et al.* (1948) believe that in salicylate treated rabbits " the lesions fail to develop because there is no antigen-antibody reaction, and that this reaction cannot take place because salicylate has prevented antigen from uniting with tissue cells." Dammin and Bukantz (1949) also pointed out that antihistamine substances did not interfere with antibody production, and it has already been shown that these substances do not reduce the incidence of vascular lesions in serum sensitized animals. Salicylates, in contrast to benadryl, have been shown to protect rabbits from anaphylactic shock, presumably by interfering with the antigen-antibody reaction, but not from histamine shock (Campbell, 1948). Although dicoumarol inhibits the antigen-antibody reaction *in vitro,* it does not inhibit the formation of the vascular lesions (Dammin and Bukantz, 1949).

The results reported above were obtained under optimal conditions, the treatment with sodium salicylate beginning on the same day as the first serum injection. Smull *et al.* (1948), who also observed a protective effect of salicylate, began to give the drug six days after the first dose of serum.

We agree with Smull *et al.* that the lesions produced in our animals do not fulfil all the rigid criteria listed by Gross, Loewe, and Eliasoph (1929) as essential in the experimental production of rheumatic fever. Aschoff-like bodies were occasionally seen, and while valvular lesions are fairly common the most striking finding is the very considerable perivascular reaction.

If these results have a clinical application to the treatment of rheumatic fever, they serve to emphasize the importance of starting treatment with sodium salicylate early in the disease. Coburn has stressed the importance of this if valvular involvement is to be minimized or avoided.

SUMMARY

The incidence of vascular and valvular lesions in the hearts of rabbits sensitized to horse serum is considerably reduced by treatment with sodium salicylate. Possible explanations of this finding are discussed.

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