Effects of Cortisol and Phenylbutazone on Experimental Arthritis and Serum Polysaccharide/Protein Ratios in Rats. (24245)

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Jasmin observed that exudate of the Murphy rat lymphosarcoma (MRLS) tumor-pouch will produce typical proliferative arthritis in rats(1). The articular lesions, which show heavy cellular infiltrations in the synovia, pannus-like proliferations and erosion of the articular cartilage(2), develop in 3-7 days and are aggravated by adrenalectomy(3). Since the polysaccharide/protein (PR) ratio of serum correlates well with clinical activity of rheumatoid arthritis(4), it was of interest to study PR ratios in rats with this type of experimental polyarthritis.

Materials and methods. Male Sprague-Dawley rats were maintained on Rockland rat diet and tap water *ad lib.* The MRLS exudate was prepared according to the method of Jasmin(2). Briefly, a tumor cell suspension was prepared and 0.5 ml was injected into a 25 ml pneumodermal pouch on dorsal side of male rats. After 7-10 days the exudate was withdrawn from the pouch, centrifuged, and either used immediately or after 1 week's storage at −5°C. One ml of this exudate was administered intraperitoneally to male rats weighing about 180 g, 24 hours after adrenalectomy. Each rat received a 0.1 mg maintenance dose of desoxycorticosterone acetate (DCA) daily. Daily therapy with cortisol or phenylbutazone, given subcutaneously in oil, was begun at time of exudate administration and continued for 5 treatments. One day after the last treatment, blood samples were taken by cardiac puncture and the animals sacrificed for macroscopic examination of the joints. Three regions were examined for swelling on each extrmity: paw, tibiotarsal or radio-carpal joint, and femuro-tibial or humero-radial joint. Severity of lesions was graded on a scale of 0 to ++++. Serum nonglucosamine polysaccharide concentrations were determined by the method of Shetlar *et al.*(5) and total serum protein was determined by the biuret method. The PR ratio of Payne *et al.*(6) was obtained from the following relationship:

$$PR = \frac{[\text{serum polysaccharide}]}{[\text{serum protein}]} \times 100.$$

Results. None of the control rats developed signs of polyarthritis, whereas 95% of the animals that received the MRLS exudate, but no therapy, and survived the treatment period, showed swelling of one or more of the sites (Table I). Only 22% of the rats that received cortisol at 1 mg were affected ($P < 0.05$). None of the animals in the 5 mg cortisol group showed any swelling ($P < 0.01$). Phenylbutazone, on the other hand, had no significant effect on incidence of arthritis ($P > 0.05$). Severity of lesions in afflicted animals was reduced significantly by cortisol treatment, but was not decreased significantly with phenylbutazone. The mortality of 28% produced by MRLS exudate was eliminated by treatment at both dose levels of cortisol, but was not reduced significantly with phenylbutazone.

MRLS exudate clearly increased serum polysaccharide levels without producing any great change in protein concentrations (Table I). PR ratios, therefore, were increased in all groups receiving exudate (Fig. 1). Cortisol treatment increased protein levels of both treated and control animals. Polysaccharide values were not reduced significantly by cortisol. Cortisol decreased the PR ratio in the rats treated with exudate and the 5 mg dose level appeared to reduce the PR ratio in the control animals. Phenylbutazone treatment produced no consistent changes in serum polysaccharide, protein, or PR ratio.

Discussion. These experiments confirm the work of Jasmin on production of polyarthritis in rats with a single injection of MRLS exudate and the inhibition of arthritic lesions with cortisol(2). In addition, it was

TABLE I. Effects of Cortisol and Phenylbutazone on Polyarthritis and Serum Changes in Rats Treated with MRLS Exudate.

Treatment	Incidence of swelling in survivors		Severity of swelling in afflicted rats	Mortality	Blood serum				
	%	Rats			Rats	Polysaccharide, mg %		Protein, %	
(A) Control									
Oil	0	0/13	0	1/14	13	150	4*	5.64	.08*
Cortisol, 1 mg	0	0/ 5	0	0/ 5	5	169	5	6.13	.06
" , 5 "	0	0/ 5	0	0/ 5	4	152	3	6.46	.06
Phenylbutazone, 20 mg	0	0/ 7	0	3/10	7	168	6	5.66	.08
(B) MRLS exudate									
Oil	95	19/20	2.5	8/28	13	228	6	5.91	.15
Cortisol, 1 mg	22	2/ 9	1.0	0/ 9	5	238	11	6.68	.11
" , 5 "	0	0/20	0	0/20	13	210	9	6.91	.15
Phenylbutazone, 20 mg	71	10/14	2.1	4/18	9	217	10	5.56	.17

* Stand. error of mean.

shown that phenylbutazone did not significantly inhibit incidence or severity of the lesions in our experiments. Phenylbutazone is weakly active, at best, in most other animal tests for anti-inflammatory activity and is metabolized rapidly in the rat(7).

Administration of MRLS exudate resulted in polyarthritis and an increase in PR ratio which was attributable to a relative increase in polysaccharide. These facts would suggest that the increased polysaccharide was associated somehow with the arthritis. Cortisol

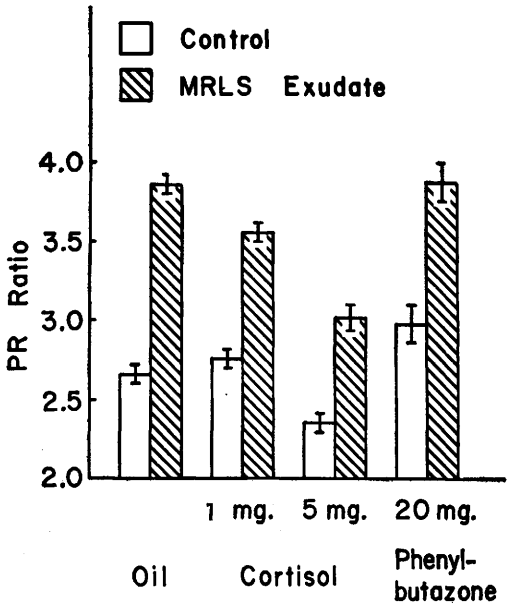

FIG. 1. Effects of cortisol and phenylbutazone on serum PR ratios in rats treated with MRLS exudate. Bars represent stand. error.

treatment reversed the shift in PR ratio mainly by increasing protein levels in the serum, suggesting that the increase in protein might have been associated with inhibition of MRLS arthritis. However, cortisol at the 5 mg level appeared to decrease PR ratio in the control rats, mainly by increasing serum protein. This might be considered the result of the "protein mobilizing" effect of cortisol(8), rather than the anti-inflammatory effect. It is of interest that both MRLS arthritis in the rat and rheumatoid arthritis in man are associated with increases in serum PR ratio and that cortisol reduces the PR ratio in both conditions. Further work will be required, however, to determine reasons for the association between inflammatory diseases and shifts in PR ratio of the serum. Winzler has discussed 5 hypotheses explaining the rise in serum glycoproteins during inflammation(9).

It is possible that tumor cells could have been transferred into the experimental animals in the tumor exudate even though the exudate had been centrifuged and no tumors were found at autopsy after 5 days. If so, this might explain the changes in PR ratio since neoplastic diseases are known to affect PR ratio(6). The observation that endotoxins increase glycoprotein levels in the serum(10) suggests another possible explanation for the shifts in PR ratio observed in our experiments. Jasmin has presented evidence that the arthritogenic agent in MRLS exudate is a microorganism growing in the tumor(2). Thus,

the shifts in PR ratio could have been the result of an infection rather than of polyarthritis, *per se.*

The animals treated with exudate plus cortisol were in much better physical condition than the animals treated with exudate alone as evidenced by appearance of the coat, activity of the animals, and mortality figures (Table I). This would indicate that under the conditions of these experiments the adrenal replacement (or anti-stress) effects of cortisol and the anti-inflammatory effects of cortisol were dominant over the pro-infective effects.

In subsequent experiments, using new batches of MRLS exudate, we were unable to reproduce the expected incidence of polyarthritis. If the arthritogenic factor in the exudate used earlier was an organism growing in the tumor as Jasmin's work suggests, perhaps we lost our "culture" during serial transplantations of the tumor.

Microscopic examination of the joints after 5 days suggested that the inflammatory reactions originated in the periarticular tissues. Conclusive proof of the site of origin will require sectioning the joints at earlier stages of the reaction and perhaps examination of serial sections. Regardless of whether the lesions originate in the periarticular or the synovial tissues, the severe inflammatory reaction produced by MRLS exudate offers a promising approach to study of inflammation and anti-inflammatory drugs.

Summary. A single injection of exudate from Murphy rat lymphosarcoma produced polyarthritis and elevated polysaccharide/protein ratios of the serum. In a later series of experiments the incidence of arthritis was much less. When arthritis was produced cortisol inhibited the incidence and severity of the arthritic lesions and partially reversed the shift in polysaccharide/protein ratio. Phenylbutazone had no consistent effect on polyarthritis or serum ratios.

1. Jasmin, G., *Rev. Can. de Biol.,* 1956, v15, 107.
2. ———, *Ann. Rheum. Dis.,* 1957, v16, 365.
3. Jasmin, G., Bois, P., Selye, H., *ibid.,* 1956, v15, 269.
4. Stidworthy, G., Payne, R. W., Shetlar, C. L., Shetlar, M. R., *J. Clin. Invest.,* 1957, v36, 309.
5. Shetlar, M. R., Foster, J. V., Everett, M. R., PROC. SOC. EXP. BIOL. & MED., 1948, v67, 125.
6. Payne, R. W., Shetlar, M. R., Bullock, J. A., Patrick, D. R., Hellbaum, A. A., Ishmael, W. K., *Ann. Int. Med.,* 1954, v41, 775.
7. Brodie, B. B., *J. Pharm. & Pharmacol.,* 1956, v8, 1.
8. Roberts, S., *J. Biol. Chem.,* 1953, v200, 77.
9. Winzler, R. J., *Determination of Serum Glycoproteins,* in D. Glick: *Methods of Biochemical Analysis,* 1955, Interscience Publ., New York, v2, 279.
10. Wöhler, F., Ghiassi, K., *Arch. Exp. Path. u. Pharmakol.,* 1957, v230, 161.

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Response to Exogenous Gonadotrophins in Absence of Dietary Protein.* (24246)

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Rats on low-protein or protein-free diets exhibit marked disturbances of the reproductive system as shown by prolonged anestrus, ovarian and accessory organ atrophy and by failure to maintain pregnancy(1,2). Estrone and progesterone at dosage levels effective in rats hypophysectomized and ovariectomized soon after breeding(3) successfully maintained pregnancy in the absence of dietary

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