If so, color may provide additional means of evaluating bacterial action and thereby be also a means of evaluating the influence of dietary factors on rumen flora.

The coefficients of correlation obtained in this study are not high, but it should be noted that they are based on relatively unrefined descriptions of the rumen. From the data presented, however, it appears that variations in development of the ruminoreticulum in general and of the rumen mucosa specifically are a structural expression of rate of growth, and possibly of capacity for growth as it is affected by general nutritional state, inherent growth impulse of the animal, or capacity of individual sheep to adapt to a functional symbiosis.

Summary. Rumina of 42 sheep, fed an adequate diet, were examined for assumed characteristics of development: total weight of reticulorumen, percent mucosa, and papillary length, width, and density. The significant correlations of rumen weight, papillary length and papillary density to rate of gain present strong supportive evidence that growth and development of the animal as a whole is related to and may be dependent upon ruminal development. Evidence is presented indicating that dietary antibiotics, chlortetracycline and oxytetracycline, may have a slight effect on mucosal development. An unexplained relationship of mucosal color and gain is presented.

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Received June 4, 1959. P.S.E.B.M., 1959, v102.

Unstable Inhibitory Mechanism in Rheumatoid Sera.* (25143)

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Serial latex fixation tests in a large group of patients with rheumatic diseases revealed a pattern of agglutination with only the euglobulin fraction of serum and not with the whole serum. Repeat freezing and thawing of whole serum in the course of these studies appeared to convert a hitherto negative whole serum latex agglutination to a positive latex whole serum agglutination. These experiences suggested an active inhibitory mechanism that might be relatively unstable.

Materials and methods. The patients were from the outpatient and inpatient services of the Maimonides Hospital. The Fraction II latex particle tests were performed by the method of Singer and Plotz(1). Squibb's lyophilized fraction II was used throughout. Euglobulin fractionation was performed using Ziff's method(2) but modified so that the precipitate was redissolved in glycine saline buffer in order to utilize the latex system. Sheep cell agglutinations were also performed by the method of Ziff and the serum, as is customary, was inactivated by heating to 56° for ½ hour before absorption. Standing sera latex particle agglutinations were performed as follows: Immediately after being separated from whole blood, serum was allowed to stand for 48 hours at room temperature. Latex particle agglutination was then carried out in the usual manner. In other procedures, 2-fold and 4-fold additions of normal fresh serum from the same patient or from healthy donors were

* Supported by grants from the N. Y. Chapter of Arthritis & Rheumatism Foundation and from the Charles Pfizer Co.

TABLE 1. Comparison of the Euglobin Latex, Standing Latex and Chemical State in 9 Patients.

Diagnosis Stage	Grade	Whole serum	SCA	Euglobin latex	Standing latex	Nodules	No. of previous neg. whole sera	Eu. + tests
2	3	Neg.	Neg.	1:224	1:320	0	2	
2	2	"	"	1:112	"	+	3	On 4th t. W.S. +
1	1	"	"	1:224	1:160	0	0	
1	2	"	"	1:56	"	+	1	
R. F.		"	"	1:224	1:80		0	
1	2	"	"	1:112	"	0	0	
? R. A.		"	"	1:28	Neg.	+	3	
3	2	"	"	1:112	"	0	2	3
? R. A.		"	"	1:56	"	0	3	

added to these standing sera and latex particle agglutination again performed.

Results. Nine sera (7 definite rheumatoid, 2 questionable rheumatoid) that had previously demonstrated a positive euglobulin latex agglutination and negative whole serum latex agglutination were studied. Allowing these sera to stand for 48 hours and then repeating the serological test on whole serum produced agglutination in the latex system in 6 sera (all definite rheumatoid) where previously there had been none. The titer of agglutination was similar in magnitude to that previously demonstrated with euglobulin if dilution differences are taken into consideration (Table I). Many normal sera from healthy volunteers were repeatedly tested in the same way and in each instance were negative.

In each of the 6 instances, the patients' own fresh serum and pooled normal human sera were added in 2-fold or 4-fold dilutions to the standing sera and the tests then repeated. However, in no instance did agglutination not occur.

Sheep cell agglutinations on the above sera were all negative.

Discussion. Previously, Ziff *et al.*,(3) and Bayles' group(4) have adequately demonstrated that by doing routine euglobulin dialysis one may add sensitivity to the serological test in rheumatoid arthritis whether one uses sensitized sheep cells or latex particles. In addition, Ziff demonstrated, as had Olsen and Rantz(5) that inhibitor could not be completely removed by euglobulin dialysis. Clark's group has suggested that complement 4′, a relatively heat stabile component of serum, was an inhibitor(6). However, heating to 56° for ½ hour should remove this factor as was done in the sheep cell test, but these tests on the same group of patients were negative. In addition, by a series of involved biochemical steps Singer(7) has suggested that complement is of no importance in performing the latex particle agglutination test.

Our studies confirm those of Olsen and Rantz and Ziff in that we have adequately demonstrated that inhibition exists clinically and serologically, the latter apart from the euglobulin fraction.

Gerber, working in Ragen's laboratory (personal communication), suggested that the alpha II fraction of serum may be an inhibitor. We cannot say at this stage in our study whether our inhibitor is an alpha II fraction, or whether this is inhibition as a result of precipitation at room temperature of gamma globulin with rheumatoid factor. Since we cannot restore inhibition, the possibility that there is an involved physico-chemical relationship, which is more of an inhibiting mechanism, rather than an active inhibitor, exists and must be studied.

The foregoing studies also suggest that an inexpensive substitute for euglobulin dialysis may be to allow the serum to stand before proceeding to dialysis. Indeed, further studies may suggest that serological tests be done only on standing sera.

Summary. Serological studies have suggested an unstable inhibitor mechanism that can be unmasked by exposure of serum to room temperature. This may be the responsible mechanism for the pattern of positive euglobulin agglutination and whole serum

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Received June 12, 1959. P.S.E.B.M., 1959, v102.

Studies of Properdin System in Human Cancer Cell Cultures.* (25144)

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The demonstration of low serum properdin titers in many patients with advanced neoplastic diseases(1-5) and the correlation of this phenomenon with diminished ability to reject homotransplants of cultivated human cancer cells(1,6) impels direct study of the effects of properdin on growth of neoplasms. The present paper reports failure of properdin to affect growth of human cancer cells in tissue culture, and the effects of incubation in tissue cultures on complement components and properdin.

Methods. Properdin (P) was prepared from normal human serum by 2 methods: adsorption onto zymosan (Z) at 17°C and subsequent elution of P from resulting P-Z complex(10), or a modification of the Cohn serum fractionation technics utilizing ethanol precipitation(16). These preparations were supplied by Dr. Louis Pillemer of Western Reserve University and Dr. Benjamin Sanders of Merck, Sharp and Dohme Labs respectively. Human serum reagents lacking P (RP) or lacking P and C'3 (R3) but containing all other components of the properdin system were prepared by adsorption with zymosan at 17°C and 37°C respectively(9,10). In studies of P prepared by Z adsorption, the same serum was the source of both P and RP. All preparations of P, RP, and R3 were tested in tissue cultures singly (*i.e.* in absence of complete P system) to eliminate preparations with innate cytoxicity. They were stored at −10°C or −60°C and thawed at room temperature immediately before use. P analyses were performed by the zymosan (hemolytic) technic of Pillemer without modification(10). All RP, R3, embryo extract, pleural fluid, and ascitic fluid preparations used had no titratable P (<1. U/ml). Parallel C'3 titrations on RP preparations in presence and absence of Z suggest that residual P was less than 0.5 U/ml. Since the tissue-culture media contained not more than 50% serum products, it follows that media without added P had residual P levels of <0.5 U/ml and probably <0.25 U/ml. For convenience these media are referred to as properdin-free. C' and C' components were assayed by the technic of Ecker, Pillemer and Seifter(17) except that 50% hemolysis end point was used instead of 100%. The hydrazine method was used for preparation of the R4. Percent hemolysis was estimated by visual comparison with standards prepared from same erythrocyte suspension used for the test. All P assays and all C' and C' component assays from a single experiment were done simultaneously using the same reagents throughout. At least one normal human serum standard (and in properdin studies, a purified properdin standard) were included in all titrations to check

* These studies supported by research grants from Nat. Cancer Inst., U.S.P.H.S., and Phoebe Waterman Fund. The authors are also greatly indebted to the late Dr. Louis Pillemer and his staff, without whose cooperation and assistance these studies could not have been initiated.