TABLE I.
Antagonism to Loosening of Corneal Epithelium After Injection of Histamine in its Dependence Upon Concentration of Antihistamines.

Amt of antihistamine per cornea in μg	Pyribenzamine	PTDA (01013)	Antistine
10.0	++		+++
5.0	++++++		++ - -
2.5	++++ -	++++	
1.0	++++	++++++++++++	- - - - - -
0.6	++++		- - - -
0.4	++++++++++++		- - - -
0.3	+++		
0.2	++++++ - - - -	++++ - - - - -	
0.1	++++ - -	++ - -	
0.08	++++ - -		
0.04	+ - - - -	- - - - - - - -	

+ Indicating removal of less than 1/3 of epithelium (protection).
- Indicating removal of more than 2/3 of epithelium (non-protection).
Each sign stands for one cornea tested. Amount of histamine injected per cornea: 2 μg.

than 1/3 with + and absence of protection with removal of more than 2/3 of the epithelium with —. It can be seen that pyribenzamine and N-(2-pyridyl)-N-(2-thenyl)-N′, N′-dimethylethylenediamine hydrochloride* provide protection in concentrations of less than 1/10 of that of histamine while the protection by Antistine requires a concentration of 2-5 times that of histamine.

* This compound is commercially designated as 01013 and is listed in the table by the initials PTDA.

Summary. The loosening of the upper layers of the epithelium of the excised bovine cornea by histamine is prevented by simultaneous injection of certain antihistamines. Three compounds found to be effective are Pyribenzamine, 01013, and Antistine.

We are indebted for the supply of the antihistamines to Dr. B. N. Craver of the Ciba Pharmaceutical Products, Inc. (Pyribenzamine, Antistine), and to Dr. K. K. Chen of Eli Lilly Co. (01013).

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Serum Polysaccharide Level in the Normal State.*

M. R. SHETLAR, JANEAL VILLET FOSTER, KEITH H. KELLY AND MARK R. EVERETT.
From the Department of Biochemistry, University of Oklahoma School of Medicine, Oklahoma City, Okla.

Several investigations concerning the polysaccharides of serum in both normal and pathological conditions have recently been published. Seibert and Atno,[1] and Seibert, Seibert, Atno, and Campbell[2] have presented results of quantitative studies using the carbazole method. Hinsberg and Merten[3] published results using an acid hydrolysis procedure while Lustig[4] *et al.* have reported several studies using an orcinol method. Nil-

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1 Seibert, F. B., and Atno, A. J., *J. Biol. Chem.*, 1946, **163**, 511.

2 Seibert, F. B., Seibert, M. V., Atno, A. J., and Campbell, H. W., *J. Clin. Invest.*, 1947, **26**, 90.

3 Hinsberg, K., and Merten, R., *Z. Klin. Med.*, 1938, **135**, 76; *Brit. Chem. Physiol. Absts.*, 1939A, III, 357.

son[5] reported elevation of the glucosamine level of serum in pneumonia. His values for average normals were: for adults 77 mg % and for fetal serum 32 mg %; pneumonia serum averaged 183 mg %. West, Clarke, Kennedy[6] reported elevations in serum glucosamine in infections, disseminated malignant disease, and sterile infarcts.

As this laboratory is engaged in a study of the animal polysaccharides in relationship to malignancy, using a tryptophane method[7] for the determination of non-glucosamine serum polysaccharides it was necessary to investigate the normal polysaccharide pattern as well as that of as many pathological conditions as possible. It is the purpose of this paper to present the results of a complete study of normal subjects. The results of our study of pathological conditions will be published at a later date.

In order to give a more complete picture, non-glucosamine polysaccharide, glucosamine, tyrosine, and total protein determinations were made on ethanolic precipitates from the same serum.

Experimental. Chemical methods. Non-glucosamine polysaccharide was determined as described in a previous publication.[7] Total protein was determined, after precipitation of the serum protein with ethanol, by a micro kjeldahl procedure.

Preliminary work with the West, Clarke and Kennedy[6] method for determining glucosamine indicated that a considerable coloration could be developed with serum hydrolysates without the acetylacetone coupling reaction, consequently the method was modified as follows to give a blank for each hydrolysate.

Reagents. 1. Absolute Ethanol. 2. 8 *N* Hydrochloric Acid. 3. 2.5 *N* Sodium Hydroxide. 4. 2.5 *N* Hydrochloric Acid. 5. Acetylacetone Solution: 0.2 ml of acetylacetone dissolved in 10 ml of 0.5 *M* Sodium Carbonate (53 g Na_2CO_3 per liter of solution). This solution must be freshly prepared before using. 6. Ehrlichs reagent: Dissolve 5 g of p-dimethylaminobenzaldehyde in 190 ml of absolute ethanol and then add 190 ml of concentrated hydrochloric acid.

Procedure. Add 1 ml of serum drop by drop to 18 ml of absolute ethanol contained in a 15 x 150 mm pyrex test tube. Allow to stand 5 minutes and centrifuge. Pour off the supernatant liquid and invert the tube to drain the excess alcohol from the precipitate. Add 2 ml of water to the precipitate and stir with a glass rod. Add 2 ml of 8 *N* HCl and hydrolyse the mixture in a boiling water bath under reflux for 4-5 hours.

Filter through Whatman No. 1 filter paper into 10 ml volumetric flasks. Make up to volume and mix. Pipet 1 to 2 ml of the solution into three 15 ml calibrated centrifuge tubes, add a drop of phenolphthalein indicator to each and carefully neutralize with 2.5 *N* sodium hydroxide. Make slightly acid by adding a drop of 2.5 *N* hydrochloric acid. Add water to bring total volume to 3.5 ml. Add 2 ml of the acetylacetone solution to 2 of the tubes and 2 ml of 0.5 *M* Na_2CO_3 to the third, which serves as a blank. Stopper lightly and place in a boiling water bath for 20 minutes.

Remove from bath and cool below 37.5°C. Make volume to 10 ml with absolute ethanol. Add exactly 2 ml of Ehrlichs solution and mix. Stopper lightly and incubate in 37.5°C oven for 45 minutes. Determine optical density in Coleman 11 Spectrophotometer at a wave length of 540 mμ. Read each sample against its repective blank. A standard containing 100 μg of glucosamine-HCl (83.4 μg glucosamine) is subjected to the same procedure with each set of samples.

Calculations

$$\frac{Kx \times 83.4 \times 10}{Ks \times V} = \mu g \text{ of glucosamine per ml of serum}$$

Kx = optical density of sample

Ks = optical density of standard

[4] Lustig, B., and Langer, A., *Biochem. Z.*, 1931, **242**, 321; Lustig, B., and Nassau, E., *Am. Rev. Tuberculosis*, 1941, **43**, 817; Novak, J., and Lustig, B., *J. Mount Sinai Hosp.*, 1947, **14**, 534.

[5] Nilsson, I., *Biocem. Z.*, 1937, **291**, 254.

[6] West, R., Clarke, D. H., and Kennedy, E. M., *J. Clin. Invest.*, 1938, **17**, 173.

[7] Shetlar, M. R., Foster, J. V., and Everett, M. R., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 125.

TABLE I.
Summary of the Non-glucosamine Polysaccharide, Glucosamine, Total Protein and Tyrosine Content of Normal Serum.

Group	No.	Polysaccharide, mg %	Polysaccharide, as % of protein	Glucosamine, mg %	Total protein, %
Fetal	15	80 (62-103)	1.41 (1.05-1.77)	48 (42-55)	5.68 (4.26-6.76)
Children (3-8 yrs)	8	105 (94-118)	1.60 (1.47-1.82)	63 (52-69)	6.59 (5.40-6.48)
Young adults:					
Males (21-32 yrs)	18	110 (93-126)	1.58 (1.26-2.02)	66 (62-78)	6.98 (6.00-7.48)
Females (22-49 yrs)	10	111 (100-125)	1.58 (1.42-1.81)	68 (61-78)	7.01 (6.26-7.35)
Aged (61-85 yrs)	15	129 (104-138)	1.79 (1.62-2.06)	81 (70-89)	7.20 (7.09-7.68)

(Figures in parenthesis indicate the high and low figures in each group.)

V = volume of aliquot of hydrolysate

Selection of Subjects. A group of young adults was selected at random from volunteer medical students who had no recent history of any pathological condition. The children's group was obtained from a local orphanage. The aged group comprised selected subjects from the outpatient clinic who had only psychosomatic complaints or fully matured cataracts, or patients selected from a State Mental Hospital. The latter patients were selected with the aid of the institutional staff who were familiar with the condition of each patient through observation over a considerable period of time. These patients were in excellent physical condition aside from mental deterioration. The fetal blood samples were drawn by needle from the umbilical vein of the cord on the placental side immediately after clamping and cutting the cord.

Results. As it is impossible to include the actual analyses of all cases, a summary of normals is shown in Table I. The original data were treated to statistical analysis, in which the averages of the different groups were compared by the following formula from Rider.[8]

$$t = \frac{X_1 - X_2}{\left(\frac{N_1 + N_2}{N + N - 2}\right)^{1/2} \left(\frac{N_1 S_1^2 + N_2 S_2^2}{N_1 N_2}\right)^{1/2}}$$

X_1 and X_2 are the averages of the respective groups, N_1 and N_2 are the number of individuals in the respective groups, and S_1^2 and S_2^2 are the variances of the respective groups. This comparison is summarized in Table II.

From the data it appears that both the polysaccharide and glucosamine associated with serum protein tends to increase with age. Levels of fetal individuals are significantly lower than those of young adults, while levels of aged persons are significantly elevated. The levels for children were found to be lower than those for young adults, but the differences were not striking. The difference is not entirely proportional to levels of the serum protein in the various groups, although fetal serums were significantly lower in protein content. When the polysaccharide content for each sample is divided by the corresponding total protein, and the data treated to statistical comparison, the "t" value for the fetal group as compared to the young adult group is 2.669, a figure which is significant at the 2% level.

Since the interrelationships between the different components are important, total protein, non-glucosamine polysaccharide, and glucosamine levels of sera from the young adults were tested for correlation. The multiple correlation coefficient for these three factors was found to be 0.601, a value which is significant at the 1% level ("t" value = 3.76). The partial correlation between protein and polysaccharide independent of glucosamine, was found to be 0.576, and the partial correlation between polysaccharide and glucosamine, independent of total protein, was found to be 0.658. Both of these figures are significant at the 1% level. The correlation between glucosamine and total protein, independent of non-glucosamine polysaccharide was found to be 0.251, a figure which failed to be significant. The reason for this lack of correlation is not immediately apparent.

If all the polysaccharides of serum are composed of equimolecular proportions of man-

[8] Rider, P. R., Modern Statistical Methods, John Wiley & Sons, New York.

TABLE II.
Statistical Comparison of Results on Normal Subjects.

Constituent	Groups compared	Difference %	t	D.F.
Total protein	Male v. female	0.03	1.280	26
Polysaccharide	" " "	1.13 mg	0.066	26
Glucosamine	" " "	1.56 "	0.648	26
Total protein	Young adults v. fetal	1.31	7.941*	41
Polysaccharide	" " "	30.37 "	9.230*	41
Glucosamine	" " "	19.13 "	12.003*	41
Total protein	Young adults v. children	0.40	2.185†	34
Polysaccharide	" " "	5.45 "	1.445	34
Glucosamine	" " "	3.75 "	1.797	34
Total protein	Young adults v. aged	— 0.22	1.707	41
Polysaccharide	" " "	—18.22 "	6.107*	41
Glucosamine	" " "	—13.53 "	7.517*	41

* Significant at the 1% level.
† Significant at the 5% level.

nose, galactose, and glucosamine, the glucosamine content divided by the non-glucosamine polysaccharide should be 0.498. The actual average figures were found to be as follows: Fetal 0.593, children 0.598, young adults 0.609, aged 0.626, weighted average 0.607.

The probability that the difference between the actual figure (0.607) and the theoretical value (0.498) could have arisen by chance is only 2.33 in 100, consequently it appears that all serum polysaccharides are not polymers of galactose-mannose-glucosamine in equimolecular proportions. There also appears to be a tendency for the ratio to increase with age, however this increase was not significant on the basis of the data obtained in this study.

Variation from time to time. In order to check the variability of serum polysaccharide in an individual, from time to time, samples were secured periodically over a period of 16 months. During this period the non-glucosamine polysaccharide varied between 110 and 122 mg %, the glucosamine between 70 and 78 mg % and the protein between 7.20 and 7.95%. This variation in polysaccharide is somewhat smaller than that recorded by Seibert and Atno,[1] and suggests that the polysaccharide level in healthy individuals is subject to about the same variation as the serum protein.

Effect of Diet. No significant difference was found in the polysaccharide levels determined on the same person fasting, 1 hour after a large meal, or 4 hours after the same meal (non-glucosamine polysaccharide levels of 115, 122 and 120 mg % respectively). Oral administration of 25 g mannose, 100 g galactose, or 50 g glucosamine caused no significant change in the polysaccharide level after 3 hours, 24 hours, or 72 hours. The polysaccharide levels were not appreciably affected by fasting (for 31 and 55 hours), by a high protein diet, by a low protein diet, or by high water intake (for 33 hours).

Stability of serum polysaccharide during storage. Data obtained from serum kept in the refrigerator for various lengths of time are recorded in the following table:

Days stored	Polysaccharide	T. protein	Glucosamine
0	120	7.20	75
1	117	—	73
2	115	—	73
3	114	—	76
7	115	7.32	74

Effect of menstrual cycle. Polysaccharide, glucosamine, and total protein determinations were made on the serum of 2 normal females at various times during the menstrual cycle (on the 2nd, 4th, 14th, 17th, and 22nd days of the cycle). Both subjects had normal 28 day cycles. The polysaccharide level varied between 111 and 118 mg % in one subject and 108 and 124 mg % in the other with no apparent correlation between the menstrual cycle and the polysaccharide level.

Summary and conclusions. Studies of serum polysaccharides were made on a total of 66

normal persons including fetal, children, young adult, and aged representatives.

The serum polysaccharide (both non-glucosamine and glucosamine) was lowest in the fetal group and highest in the aged representatives, the children and young adult groups being intermediate, thus showing a tendency to increase with age.

Concentrations of serum polysaccharide (both non-glucosamine and glucosamine) for young adults were significantly higher than those of fetal serums, and significantly lower than for the aged. The total protein for young adults was significantly higher than in fetal sera. Significant positive correlations were found between non-glucosamine polysaccharide and total protein and also between non-glucosamine polysaccharide and glucosamine.

In the same individual, non-glucosamine serum polysaccharide and glucosamine were subject to about the same variation as serum protein over a period of sixteen months. Small variations in diet appeared to have little effect. No appreciable changes occurred in serum polysaccharides during the menstrual cycle.

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Failure of Trypan Red to Protect Against Certain Neurotropic Viruses ("M.M." and Russian Spring-Summer Encephalitis).*

W. McD. HAMMON, R. B. AIRD AND GLADYS SATHER.

From the George Williams Hooper Foundation for Medical Research, and the Division of Neurology of the University of California, San Francisco.

Investigations by Aird[1] on brilliant vital red and by Aird and Strait[2] on trypan red have shown these supra-vital dyes to affect the distribution of intravenously injected cocaine within the central nervous system. This was presumably due to an alteration of the permeability of the blood-brain barrier since the amount of cocaine reaching the cerebral cortex of cats receiving the dyes was decreased by 31% as compared with the controls not receiving the dyes. As a result of these studies and others, the question was raised as to the possible protective effect of these substances against infection of the central nervous system by neurotropic viruses. Due to the interruption of our studies by the late war we were unable to engage in experimental work along these lines.

Wood and Rusoff[3] claimed to demonstrate the protective effect of trypan red on the "M. M." virus.[4] Unfortunately, Wood and Rusoff inoculated the dye repeatedly by the intraperitoneal route and subsequently inoculated the virus by the same route. It has been the general experience of bacteriologists and immunologists that a local, non-specific protection against most infectious agents is set up in the peritoneal cavity by many types of inert substances when given previously by the same route.

Further studies on the possible protective effect of the supra-vital dyes against virus infections of the central nervous system, therefore, appeared indicated. Considering the mode of action of the dyes, it seemed wise to select viruses capable of infecting the central nervous system when inoculated by a peripheral route, as opposed to viruses requiring direct inoculation into the central

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1 Aird, R. B., *Arch. Neurol. and Psychiat.*, 1939, **42**, 700.

2 Aird, R. B., and Strait, L., *Arch. Neurol. and Psychiat.*, 1944, **51**, 54.

3 Wood, H. G., and Rusoff, I. I., *J. Exp. Med.*, 1945, **82**, 297.

4 Jungeblut, C. W., and Dalldorf, G., *Am. J. Pub. Health*, 1943. **33**, 169.