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Correlation of Toxicity of Normal Globulins with Increased Partition of Mid and End-Piece of Alexin.

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Bordet[1] has demonstrated that the intravenous injection of globulins from fresh, normal guinea pig serum into guinea pigs is followed by extreme shock and death of the animal, usually within 3 or 4 minutes. In 1925, together with Bordet, further study was made of this phenomenon.[2] In this study it was found that the toxicity of the globulin fraction of guinea pig serum for guinea pigs was destroyed when heated at 70° C. for 1/2 hour; that the toxicity of the globulin fraction remained intact when heated at 60° C. for the same length of time; that the leucocyte count of the animal was decreased to about 1/2 following injection; that the temperature of the animal increased 3 to 4 degrees and there was a delay in the coagulation time of the blood following injection, in some cases as long as 24 hours. At the same time it was shown that injections of globulins intraperitoneally or subcutaneously into guinea pigs were innocuous.

Later, in collaboration with Zunz[3], it was demonstrated that following the intravenous injection of globulins from guinea pig serum into guinea pigs there was, in some cases, a definite lowering of the surface tension of the plasma. This lowering of the surface tension it was thought might be due to the presence of hemoglobin in the plasma, and this, in turn, was accounted for by the alkalinity or acidity of the injected material. Zunz[4] has shown that the presence of hemoglobin notably lowers the surface tension of both water and plasma.

Our method of precipitating the globulins from guinea pig serum consists in diluting with 20 volumes of water and passing CO_2 gas through the mixture several times. After centrifugalization for 10 minutes the supernatant fluid is removed and the precipitate is dissolved in physiological salt solution to the volume of the original serum employed. The mixture is then made slightly alkaline with Na_2CO_3 to insure greater solubility. Such preparations are usually toxic for guinea pigs, in doses ranging from 1 1/2 cc. to 4 cc. when given intravenously, and usually produce shock within a few minutes, followed by death of the animal. It has been our

experience that not all such preparations are toxic and this we have been unable to explain. However, upon the addition of lipoids to the 20 volumes of distilled water (lipoids from 1 cc. of syphilitic antigen after evaporation of alcohol for each 5 cc. of serum) it was found that such preparations were invariably toxic. In a few instances following the injection of such material we have found at autopsy changes in the lungs which closely resemble true anaphylactic shock.[3] This, however, is by no means the rule, as the phenomenon as first described by Bordet is quite different.

The role of lipoids and their effect upon the biological reactions of serum is little understood. Why should the addition of a small amount of lipoid to the distilled water before CO_2 precipitation always insure a toxic product, when without this addition various samples of globulins from sera are found to be non-toxic, although their preparation has been precisely the same in every instance? We might conceive of lipoid in this instance as an adsorption compound coating the protein molecule, followed by a more rapid and complete precipitation. The pH of our preparations are quite uniform, though the isoelectric point might conceivably be altered somewhat by our method. Powis[5] and Northrop[6] have observed that the critical potential of a suspension of sheep cells is raised by the presence of paraffin oil. Northrop regards this effect as closely analogous to the more general one of "protective colloids."

Since we are dealing here with the mid-piece of complement (end-piece is not toxic) it is suggested that the uniform toxicity of mid-

TABLE I.

Effect of addition of lipoid to distilled water before precipitation of mid-piece from normal guinea pig serum with CO_2 gas.

5% susp. of sheep cells	Salt sol.	1-200 sensitizer	Alexin 1-10	With lipoid		Without lipoid		Reading	
				Mid-piece	End-piece	Mid-piece	End-piece	30" 37° C.	12 hrs.
.05 cc.	1 cc.	.2 cc.	.1 cc.	—	—	—	—	CH	CH
"	"	.3 cc.	"	—	—	—	—	CH	CH
"	"	—	"	—	—	—	—	N	N
"	"	.2 cc.	—	.1 cc.	—	—	—	N	N
"	"	"	—	.2 cc.	—	—	—	N	N
"	"	"	—	—	.1 cc.	—	—	N	PH
"	"	"	—	—	.2 cc.	—	—	N	CH
"	"	"	—	—	—	.1 cc.	—	N	PH
"	"	"	—	—	—	.2 cc.	—	N	PH
"	"	"	—	—	—	—	.1 cc.	N	PH
"	"	"	—	—	—	—	.2 cc.	N	CH
"	"	"	—	.1 cc.	.1 cc.	—	—	PH	CH
"	"	"	—	.2 cc.	.2 cc.	—	—	CH	CH
"	"	"	—	—	—	.1 cc.	.1 cc.	PH	CH
"	"	"	—	—	—	.2 cc.	.2 cc.	CH	CH

piece, obtained when lipoids are added to the first distilled water, might be due to a more complete separation of mid-piece from end-piece than one ordinarily gets with the usual methods employed. This hypothesis has been tested in a hemolytic system to determine if such is the case. The following protocol will serve to illustrate the results obtained in a series of experiments:

TABLE II.

Effect of addition of Na_2CO_3 to final solution of mid-piece prepared with and without lipoids.

5% susp. of sheep cells	Salt sol.	1-200 sensitizer	Alexin	With addition of lipoid and Na_2CO_3		Without lipoid but with Na_2CO_3		Reading	
				Mid-piece	End-piece	Mid-piece	End-piece	30 min.	12 hrs.
.05 cc.	1 cc.	.2 cc	—	.1 cc.	—	—	—	N	N
"	"	"	—	.2 cc.	—	—	—	N	N
"	"	"	—	—	—	.1 cc.	—	N	N
"	"	"	—	—	—	.2 cc.	—	N	N
"	"	"	—	.1 cc.	.1 cc.	—	—	PH	CH
"	"	"	—	.2 cc.	.2 cc.	—	—	CH	CH
"	"	"	—	—	—	.1 cc.	.1 cc.	PH	CH
"	"	"	—	—	—	.2 cc.	.2 cc.	CH	CH

CH—Complete hemolysis. PH—Partial hemolysis. N—No hemolysis.

In Table I after 12 hours there was no hemolysis in the tubes which had received mid-piece precipitated from guinea pig serum, with addition of lipoids to the 20 volumes of distilled water before CO_2 gas precipitation. The tubes which had received mid-piece precipitated without addition of lipoids, showed partial hemolysis. End-piece in both cases after 12 hours produced complete hemolysis. This would seem to indicate that where lipoids are added to the distilled water used for diluting the serum before CO_2 precipitation there is a more complete separation of the mid-piece from end-piece. That is, there is little or no end-piece carried down with mid-piece, or at least a quantity below that which is necessary to act with mid-piece in producing hemolysis. Whether this is due to the rapidity of precipitation, or to a selective action of the lipoids for the globulin molecules as opposed to the albumin, we are not certain. In this experiment the final solution of mid-piece was on the acid side of neutral. Table II illustrates, at least in several experiments, of which this is a type, that when the pH of the mid-piece is slightly on the alkaline side, either with or without the addition of lipoids in their preparation, there is apparently complete separation, though these results are by no means regular. This again may

simply indicate that there is not sufficient end-piece present to act with mid-piece in bringing about hemolysis and not that there is a complete absence of end-piece. It is, of course, apparent that these experiments are only rough titrations of hemolysis.

On the whole, it is indicated by these experiments that probably there is no greater separation of mid-piece from end-piece brought about by the presence of lipoid in the distilled water dilution of the serum than there is without lipoid. If there was greater separation we would have expected a difference in Table II between the two preparations. This did not occur. The uniform toxicity of globulins from normal guinea pig serum (prepared with lipoid) for guinea pigs is not to be explained upon this basis. It is suggested that the character of the precipitate may in some manner be modified and that the resulting solution-suspension of globulins is found in a different "colloidal state" which contributes to the toxicity of the preparation.

[1] Bordet, J., *Compt. rend. Acad. sc.*, 1924, clxxix, 243.

[2] Bordet, J., and McKinley, E. B., *Compt. rend. Soc. biol.*, 1925, t. xcii, 762-764.

[3] McKinley, E. B., and Zunz, Edgard, *Compt. rend. Soc. biol.*, 1925, t. xciii, 459.

[4] Zunz, Edgard, *Bull. Soc. roy. Sc. med. et nat.*, Brussels, 1906, t. lviv, 187-203.

[5] Powis, F., *Z. physik. chem.*, 1914-15, lxxxix, 179, 186.

[6] Northrop, John H., *J. Gen. Physiol.*, 1924, vi, 603.

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Effect of High Voltage Cathode Rays on Rickets and on the Activation of Cholesterol.

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It has been shown repeatedly that ultraviolet radiation brings about healing of rickets and also that irradiation of cholesterol by ultraviolet light renders it antirachitic. Recently, one of the authors[1] has developed a means for producing high voltage cathode rays outside of the generating tube. It seemed interesting to study the effect of these rays on experimental rickets in rats, first when applied directly, and second when used for the activation of cholesterol.

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