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Further studies on the physiological action of non-specific antigens prepared from shattered hemo-proteins.

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During the past decade, one of us (Brooks) has made extensive investigations of new methods for combating infections. In the course of this study, the following hypothesis was formulated:[1] In the blood and blood-forming organs are found the various chemical structures, cellular or otherwise, which are responsible for the production of the antibodies, formed in the immunity response to infection. Consequently the blood should be good material from which to isolate immunifacient antigen bodies.

Acting upon this theory, a protein was carefully prepared by shattering the fibrin of ox-blood with hydrochloric acid and pepsin. The residue was fractionated by precipitation with ammonium sulfate, retaining the lower secondary proteoses, which are the most active components. These proteoses, he found, were multiple in number and varied in nature, but their very complexity may explain their service in combating infections of different etiology.

A host of workers has searched for a protein which would be efficient therapeutically, and yet rid of the disagreeable concomitants of protein action; namely, fever, chills, sweating, etc. The above mentioned proteose seems to fill this requirement. Brooks and Stanton[2] noted the absence of the so-called "characteristic protein reaction" and at first, on this account, were inclined to doubt the efficacy of this shattered hemo-protein antigen, but this view was dispelled by the remarkable success of this protein in a large series of cases of acute and chronic arthritis. The general opinion heretofore is best expressed by quoting Petersen[3]:

[1] Brooks, C., Non-specific protein antigens prepared from shattered hemo-proteins, *Science*, 1919, xlix, 196.

[2] Brooks, C., and Stanton, F. M. Non-specific hemo-protein antigen for the treatment of arthritis, *N. Y. Med. J.*, 1919, cix, 452.

[3] Petersen, W. F., Protein therapy and non-specific resistance, New York, 1922.

"There is a probability that . . . the positive phase or mechanism of recovery after non-specific injections is a function, or at least very closely related to the degree of the negative phase or the intensification of the disease process that is clinically manifest in the reaction of the patient. Just as in local foci of disease a marked augmentation of the inflammation, both after specific vaccine injections or non-specific injections, is followed as a rule by clinical improvement, so in the general infections a relatively severe reaction is more frequently followed by an abortive recovery than when the reaction is very mild or absent."

But the experience of Brooks and Stanton indicates that the depressing phase of the process is not an essential feature of antigen action, as exemplified by their results with shattered hemoprotein. The interpretation of other investigators, that the "reaction" is causal in promoting recovery, is probably a misconception, based upon the complicating factor of concurrent "reactions" with the injections of the proteins previously used. In fact, it is quite conceivable that the "reaction" often seen in foreign protein therapy is due to the action of *a certain group of proteins*, and the beneficial action is due to *an entirely different group of proteins,* and therefore if the two groups are successfully separated we may have, on the one hand, *the group of toxic proteins* which gives a marked "reaction" but has no therapeutic value, and on the other hand, *the group of therapeutic proteins* which has no toxic action, but acts therapeutically.

This is not the only difference between this hemo-proteose and other proteoses. Whereas the latter, upon injection, induce a primary leucopenia of varying duration and intensity, and a subsequent secondary leucocytosis, Brooks[4] found that his hemo-proteose by its leucotonic action, stimulated leucopoiesis immediately upon injection and that after several hours a well marked leucocytosis was apparent, without the primary intervening leucopenia. This is evidently a desirable and valuable pharmacological action.

With dogs, guinea pigs and rabbits as the experimental objects, we have attempted to produce sensitization to subsequent hemoproteose injections, but upon repeated injections of the protein, employing varying doses and intervals, we find that the animals

[4] Brooks, C., The effect of shattered hemo-protein on the colorless blood corpuscles, *Am. J. Physiol.*, 1919, xlix, 127.

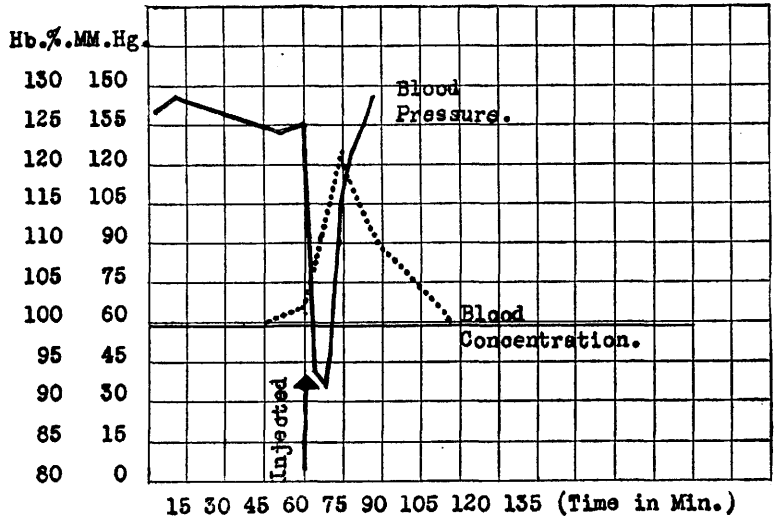

Chart One. Dog C. (0.2 gm. Brook's Hemo-proteose injected intravenously per kgm. body weight of dog. Weight of dog 10.4 kgms.).

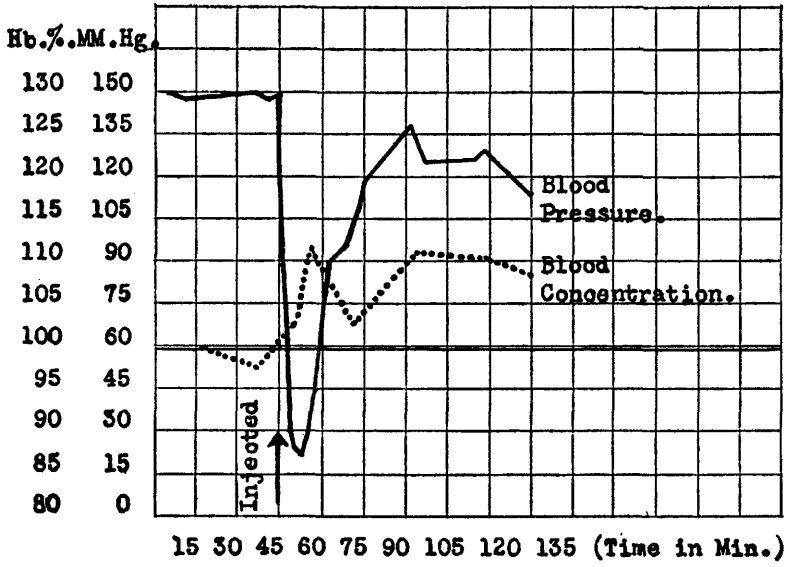

Chart Two. Dog B. (0.2 gm. Witte's Peptone injected intravenously per kgm. body weight of dog. Weight of dog 14.5 kgms.).

are not sensitized, but are more tolerant to successive doses. This apparent increased tolerance harmonizes with the desensitization noted clinically when the hemo-protein is employed. It also suggests that the hemo-protein could advantageously be administered in gradually increasing doses in certain cases. We are planning to test the possibility of sensitization in guinea pigs by intracranial injections.

How can the absence of these undesirable effects in the use of this new proteose be explained? Is it weaker than other proteins, and thus the dosage used too small to evoke a response? Experimental evidence is against this view. First, the therapeutic index is at least equal to that of other proteins. Second, the pharmacological action of this proteose is comparable to the action of other proteoses.

In the present paper we have attempted to compare the physiological activities of Witte's peptone with the action of this new hemo-proteose on blood concentration and blood pressure. Underhill and Ringer[5] have demonstrated that in dogs an increased blood concentration accompanies the shock induced by either Witte's peptone or by deuteroproteoses prepared from egg white. Our proteose, prepared from blood fibrin, will, with an equal dosage, produce a similar and equivalent physiological change. However the blood concentration returns to normal more quickly with our proteose than when Witte's peptone is used. This may be accounted for by the fact that Witte's peptone contains a large proportion of primary proteoses and peptones, as well as secondary proteoses, and primary proteoses are generally more toxic than deuteroproteoses.

The hemoglobin percentage is taken as the measure of the relative blood concentration of the dog. The values given in the charts are relative ones, the hemoglobin being expressed in percentage of the initial readings rather than the absolute value. The dosage employed in both series was 0.2 gm. of the protein per kilogram of body weight. The proteins were rapidly injected intravenously. The intravenous method is the route par excellence for the therapeutic use of hemo-proteose, and has been the method of clincal choice in practice. This obviates the increasing

[5] Underhill, F. P., and Ringer, M., The relation of blood concentration to peptone shock, *J. Pharm. and Exp. Ther.*, 1923, xix, 163.

TABLE I.

The Toxicity of Brooks' Hemo-proteose for Albino Rats. (Intraperitoneal Injections.) Date, February 19, 1924.

No. of rat.	Wt. in gm.	Dosage in mg. per 100 gm. of rat.	Liquid dose per 100 gm. of rat.	Total no. cc. injected.	Total wt. of protein injected in gm.	Time of injection A. M.	Time interval until death in minutes.	Time of death A. M.	Symptoms.
1.	191	5	0.5 cc. of 1%	0.95 (1%)	0.0095	9:21			None
2.	177	10	1.0 cc. of 1%	1.77 (1%)	0.0177	9:24			Cyanosis
3.	234	20	2.0 cc. of 1%	4.68 (1%)	0.0468	9:26			Cyanosis
4.	250	40	0.8 cc. of 5%	2.00 (5%)	0.100	9:27			Cyanosis
5.	277	60	1.2 cc. of 5%	3.32 (5%)	0.1662	9:28			Cyanosis
6.	217	75	1.5 cc. of 5%	3.25 (5%)	0.1627	9:29			Cyanosis
7.	181	100	2.0 cc. of 5%	3.62 (5%)	0.181	9:31			Convulsions
8.	229	125	1.25 cc. of 10%	2.86 (10%)	0.2862	9:33	24	9:57	Convulsions
9.	190	150	1.50 cc. of 10%	2.85 (10%)	0.285	9:36	32	10:08	Convulsions and shock
10.	254	175	1.75 cc. of 10%	4.44 (10%)	0.4445	9:38	17	9:55	Convulsions and shock
11.	197	200	2.00 cc. of 10%	3.94 (10%)	0.394	9:39	11	9:50	Convulsions and shock
12.	203	225	2.25 cc. of 10%	4.97 (10%)	0.4567	9:41	13	9:54	Convulsions and shock
13.	231	250	2.50 cc. of 10%	5.77 (10%)	0.5775	9:45	8	9:53	Convulsions and shock
14.	233	275	2.75 cc. of 10%	6.40 (10%)	0.640	9:47	20	10:07	Convulsions and shock

non-absorption of the proteins when injected subcutaneously, which is caused by precipitation at the locus of injection.[6]

At present the quantity of proteose used for intravenous injections for the human varies from 15 to 40 mg. repeated every forty-eight hours. The administration of this small quantity is quite safe. The lethal dose for the albino rat is 125 mg. per 100 gm. body weight. Applying the lethal dose for the rat to man, 87.5 gm. would be the lethal dose for a 70 kilo man. This is more than two thousand times the largest therapeutic dose employed. (See Table I.)

CONCLUSIONS

1. The so-called "reaction" seen in protein therapy is not at all necessary in order to secure beneficial therapeutic action.

2. Shattered hemo-protein has qualities which make it useful in therapy: it is desensitizing; it acts as a non-specific immunizing antibody; and it is of low toxicity, producing almost no "reaction" in therapeutic doses.

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The differentiation of two distinct types of phagocytic cells in the spleen of the rabbit.

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It has come to be the accepted opinion that the phagocytic cells of the tissues, which have been designated as *clasmatocytes, macrophages* or *histiocytes*, and the large mononuclear elements of the blood, the so-called *monocytes* or *transitionals,* are cells which are very closely related if not identical. The majority of observers consider them to be derivatives of the reticulo-endothelial apparatus or of endothelial cells in general; indeed one of us (Sabin[1]) has presented the view that they are identical cells

[6] Opie, E. L., The fate of injected protein in an animal immunized against it, PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 162.

[1] Sabin, F. R., *Contrib. to Embryol.*, 1920, ix, No. 36, Carnegie Institution of Washington, Publication No. 272.