6415

Temperature Reaction Following Intravenous Injections of Proteins of Navy Bean with Special Reference to Hemagglutinating Fraction.*

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Hemagglutinin preparations from seeds of *papilonaceae, i. e.,* beans, peas, lentils and vetch, were first made by Landsteiner and Raubitschek.[1] Because these substances did not produce death when relatively large amounts were injected intraperitoneally into mice and guinea pigs, they were designated as "non-toxic". Since the hemagglutinating fraction from navy beans has been used as an agent for ridding anti-sera of suspended erythrocytes[2] and as it is capable of being highly purified without being rendered insoluble,[3] it is of interest to learn whether it causes the production of a fever upon injection. The results here reported give the effect upon the rectal temperature curve of rabbits of intravenous injections of the hemagglutinin prepared from the navy bean.

Fifteen rabbits were used for the injections which were made in 5 cc. volumes into the marginal ear vein. The materials tested for pyrogenicity were dissolved in sterile redistilled water prepared by the method outlined by Seibert.[4] The temperatures of the animals, taken by a Faichney rectal thermometer, were recorded at hourly intervals for 7 or more hours. As was found by Seibert and Mendel,[5] the range of temperature variations for individual rabbits was fairly constant for the normal uninjected animals. (Plate I, a. b. c. and d.) The negative reactions of the animals injected with the water used as a solvent are shown by the curves of Plate II, a, b, c, and d. The temperature reactions resulting from the injection of a purified preparation of the hemagglutinin from the navy bean were found to be even stronger than those resulting from injections of egg albumin and peptone solutions. (Plate III.) The preparation

* The data reported in this paper are taken from the thesis presented by Rosemary Anne Murphy in partial fulfillment of the requirements for the degree of Master of Arts, Wellesley College, 1931.

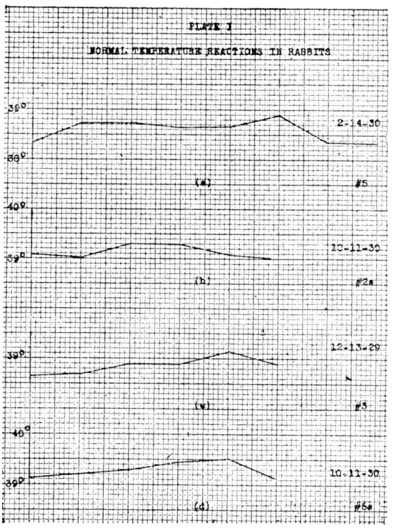
1 Landsteiner and Raubitschek, *Centbl. Bakt.*, 1907, **45**, 660.

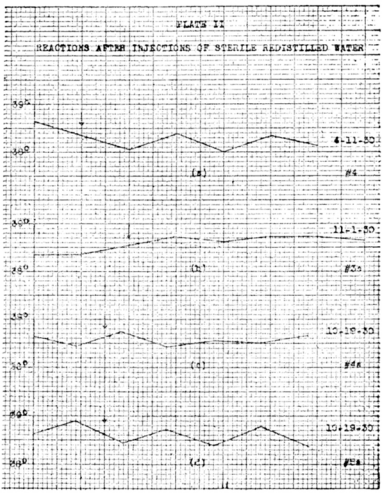
2 Dorset and Henley, *J. Agric. Research*, 1916, **6**, 333.

3 Goddard and Mendel, *J. Biol. Chem.*, 1929, **82**, 447.

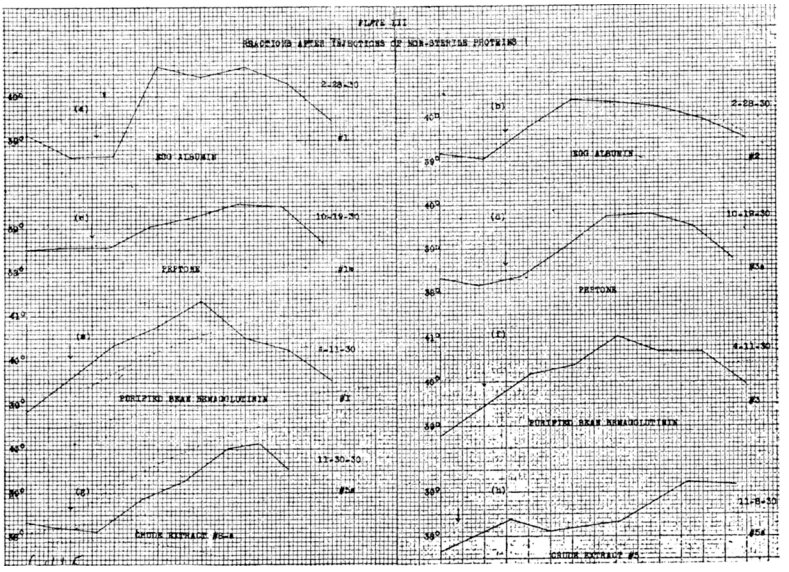
4 Seibert, *Am. J. Physiol.*, 1923, **67**, 90.

5 Seibert and Mendel, *Am. J. Physiol.*, 1923, **67**, 83.





of hemagglutinin used had been stored for 5 years in powder form and carefully protected from moisture. No attempt had been made either to prepare or to keep it in a sterile condition. Freshly prepared crude extracts of the hemagglutinin of the navy



bean were found to be on the whole less pyrogenic than the highly purified material which had been stored. Sterile redistilled water was used in making these extracts. After having been washed in the non-pyrogenic water, the beans were ground in a sterile mortar and

TABLE I.
Temperature Reactions Following Intravenous Injections of Proteins.

Date	Protein Injected	Amt. Injected mgm.	No. of Animals	Temperature Variation °C. +	Temperature Variation °C. —	Solvent* Water
2- 7-30	Egg Albumin	5	4		0.55	B
	" "	5	5	1.11		B
2-28-30	" "	5	1	2.01		C
	" "	5	2	1.33		C
3- 7-30	Purified Hemagglutinin	5	4	2.45		B
	" "	5	5	2.22		B
4-11-30	" "	5	1	2.50		D
	" "	5	3	2.23		D
10-19-30	Peptone	500	1a	1.05		AA
	"	500	3a	1.60		AA
11- 8-30	Crude Extract No. 5	—	2a	0.84		BB
	" " "	—	4a	0.77		BB
	" " "	—	5a	1.56		BB
	" " "	—	6a	0.78		BB
11-30-30	" " No. 8-a	—	5a	2.00		DD1
	" " "	—	6a	0.94		DD1

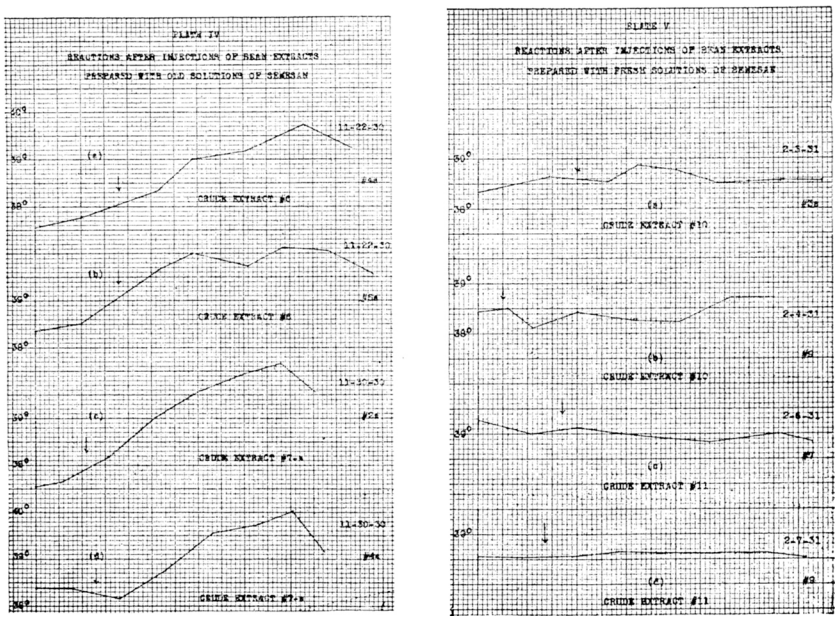
* These are non-pyrogenic, distilled waters, prepared according to the method of Seibert and Mendel.[9]

soaked in 4 times their weight of water for 15 hours at 5°C. The supernatant fluid, which was poured off and injected, contained some starch grains as well as proteins and traces of other substances extractable from the bean. Table I gives the temperature variations following these injections, compared with those resulting from injections of other protein solutions mentioned above.

Working on the assumption that a protein at its source would be free from bacterial contamination, an attempt was made to obtain a non-pyrogenic protein solution by extraction of the beans after the outer coating had been sterilized. A commercial seed sterilizer known as Du Bay Semesan was used. Its composition is given on the package as 30% hydroxymercurichlorophenol and 70% inert ingredients. A 0.25% solution of this material was used. The beans were soaked in Semesan for one hour to sterilize the seed coat. The poison could then be completely removed by washing the beans in the sterile redistilled water. The hydroxy-phenyl group of the sterilizer was readily detected by Millon's reagent for this grouping. Washings at hourly intervals were continued for some 5 hours after a negative Millon's test resulted.

From beans sterilized in accordance with the method indicated and extracted with non-pyrogenic water, extracts were obtained which did not cause fever upon injection. Good results were obtained, however, only when a *freshly prepared* solution of sterilizer

was used. Semesan solutions a week or more old were ineffective in preventing strongly positive fever reactions when the extracts were injected. Plates IV and V draw a comparison between the



PLATES I-V.

The date of experiment and the number of animal are found with each curve. Arrows indicate time at which injections were made. One cm. abscissa equals 30 min. One cm. ordinate equals 0.5°C.

temperature reactions following the use of old Semesan solutions, with those from beans sterilized with freshly prepared solutions.

To eliminate the possible criticism that the non-pyrogenicity of the extracts might be ascribed to the fact that the protein present was insufficient in quantity to produce a fever, analyses were made of the amounts of protein present in 5 of the crude extracts. Nitrogen was determined by a modified *Folin-Farmer Micro-Kjeldahl* method.[6] The nitrogen figure was multiplied by the factor 6.25 for the protein estimation. The amounts found (13-17 mg.) varied considerably as a result, probably, of differences in the extent to which the beans were ground in the process of preparing the samples. In every case, however, the amount injected far exceeded the weights of proteins injected in the fever-producing solutions listed in Table I with the exception of the peptone solution. They also exceeded

[6] Hall, unpublished data.†

† Modification of the Folin-Farmer Micro-Kjeldahl method.

the amounts which Seibert and Mendel injected[7] to produce characteristic fevers.

The results give additional support to the conclusion of Seibert[4] that bacteria are responsible directly or indirectly for the production of fever upon the introduction into the circulation of substances foreign to it. Her conclusions came as the result of an exhaustive study of the fever-producing substances in distilled waters. The work of Rademaker[8] likewise confirms her hypothesis. Barkan and Nelson[9] studied the cause of the febrile reactions following injections of milk and concluded that bacteria or products of bacterial action and not the proteins of the milk were pyrogenic. The work reported here indicates that the proteins of the navy bean are not pyrogenic *per se* but that they are easily contaminated. Injected extracts prepared from navy beans produce a slight or a strong fever reaction, depending upon the degree of bacterial contamination.

Summary. A method is presented for preparing a strongly hemagglutinating, non-pyrogenic extract from navy beans. This extract is high in protein content. The method demonstrates that the proteins of the bean which may be extracted by water are not fever-producing unless contaminated by bacteria or products of bacterial action.

6416

Oxygen Consumption by Acidified Tissues.

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Amberson, Armstrong and Root[1] have described a curious phenomenon; the persistence of oxygen consumption without carbon dioxide production in acidified tissues. *Fundulus* eggs, for instance, continued to take up some 7 to 14% of the oxygen which they had previously been consuming in normal respiration. This residual oxygen-uptake persisted after neutralization of the acid, and was not affected by KCN or by high temperatures.

In recent experiments[2] on the respiration and respiratory quotient

[7] Seibert and Mendel, *Am. J. Phiysiol.*, 1923, **67**, 105.

[8] Rademaker, *Ann. Surg.*, 1930, **92**, 195.

[9] Barkan and Nelson, *J. Am. Med. Assn.*, 1924, **82**, 190.

[1] Amberson, Armstrong and Root, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 31.

[2] Needham, *Proc. Roy. Soc. London*, B, 1932.