REDUCING THE PYROGENICITY OF CONCENTRATED PROTEIN SOLUTIONS

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Heretofore the removal of bacterial pyrogens from concentrated protein solutions has not been a practical procedure. Zittle and coworkers (1945) in a study of pyrogen removal from protein hydrolyzates found that although Republic S-3 pads effectively removed pyrogens when the solute was of small molecular weight (acid hydrolyzates), these pads were effective only with tremendously expanded filter pad surface area when the solute was of high molecular weight (enzymic hydrolyzates). Similarly, Republic S-3 pads or any of the Seitz type filter pads described as useful for pyrogen removal from crystalloid solutions (Co Tui and Wright, 1942) or from antibiotics (Tonnesen and Vesterdal, 1946) have proved ineffectual in our hands for the removal of pyrogens from protein solutions. The use of charcoal, which has been described as a pyrogen adsorbent (Howard and Spooner, 1946), presents great problems because of the extreme difficulty of its removal from protein solutions.

However, using a new type of Seitz pad, Republic Filter's K-6 and S-6 pads, developed for plasma filtration during the war (Rane and Baldwin, 1945), and an ionic exchange agent, "decalso,"² we have been successful in reducing the pyrogenicity of a variety of protein solutions in a manner which permits practical application.

Pyrogen removal, by the technique to be described, has been accomplished with serum, plasma, and hemoglobin solutions ranging from 6 to 12 per cent protein and with normal human serum albumin solution containing 25 per cent protein. Several solutions of each type have been treated successfully. Pyrogen removal is a function of the degree of pyrogenicity and the concentration of the protein solution, and the proper relationship of the amount of "decalso" and filter pad area to these variables. This latter relationship must be evaluated for each solution to be treated, often by preliminary trials. Thus the pyrogenicity of some protein solutions may be reduced by treatment with "decalso" alone, others by filtration alone, whereas some solutions require treatment with increased quantities of "decalso" plus filtration. The data have been selected to illustrate these points.

PROCEDURE

The pyrogen tests described were all performed by the procedures prescribed by the National Institute of Health for pyrogen tests of biological products

¹ With the technical assistance of Lois Priester.

² Manufactured by the "Permutit Co.," New York, New York. Our attention was first drawn to the possible usefulness of "decalso" for pyrogen removal by Dr. R. A. Phillips, Hospital of the Rockefeller Institute.

(Minimum Requirements for Pyrogen Tests on Biologic Products from Blood Serum, Nov. 19, 1945, National Institute of Health).

The pyrogen-free water used in these experiments was prepared by double distillation through Barnstead stills. The water was checked repeatedly for pyrogenicity. All equipment coming in contact with the solutions was carefully cleaned and rinsed with pyrogen-free water before use.

"Decalso" itself occasionally contains a pyrogenic substance. This can be removed by stirring the "decalso" with ten times its weight of a 0.5 per cent solution of sodium carbonate in pyrogen-free water, decanting the supernatant, and washing the "decalso" with pyrogen-free water until the washings are neutral to litmus. All "decalso" used in these experiments was so treated.

In experiments in which the effects of "decalso" alone were to be examined the relatively ineffective S-3 pads were used for sterile filtration.

REMOVAL OF PYROGEN FROM HEMOGLOBIN SOLUTION WITH "DECALSO"

A sterile, pyrogenic solution containing approximately 7 per cent hemoglobin gave the following rises above the starting temperatures in the routine pyrogen test.

<i>Rabbit</i>	<i>1 hr</i>	<i>2 hr</i>	<i>3 hr</i>	<i>Max rise</i>
1	1.4 C	1.5 C	-0.3 C	1.5 C
2	1.0 C	0.8 C	0.2 C	1.0 C
3	0.5 C	1.1 C	0.3 C	1.1 C

Two hundred ml of this solution were shaken with 6 g of "decalso" and allowed to stand overnight in the cold (2 C). The solution was then filtered through an S-3 pad, using 100 sq inches of pad per liter of solution. The pyrogenicity of the solution was greatly reduced as shown in the following test.

<i>Rabbit</i>	<i>1 hr</i>	<i>2 hr</i>	<i>3 hr</i>	<i>Max rise</i>
1	0.1 C	0.3 C	0.0 C	0.3 C
2	0.3 C	0.2 C	-0.2 C	0.3 C
3	0.1 C	0.0 C	0.1 C	0.1 C

Five liters of this hemoglobin solution were shaken with 30 g of "decalso" per liter. After standing 4 hours at 2 C, the solution was filtered through an S-3 pad, using a greatly reduced pad surface area, i.e., about 8 sq inches per liter of solution, thereby eliminating for all practical purposes the effect of filtration. It will be seen that the "decalso" itself had an appreciable effect.

<i>Rabbit</i>	<i>1 hr</i>	<i>2 hr</i>	<i>3 hr</i>	<i>Max rise</i>
1	0.9 C	0.9 C	0.9 C	0.9 C
2	0.2 C	0.5 C	0.4 C	0.5 C
3	0.0 C	0.5 C	0.5 C	0.5 C

REMOVAL OF PYROGEN FROM PLASMA WITH S-6 FILTRATION AND WITH "DECALSO"

A sterile plasma pool was found to be pyrogenic as shown by the following test.

<i>Rabbit</i>	<i>1 hr</i>	<i>2 hr</i>	<i>3 hr</i>	<i>Max rise</i>
1	0.3 C	0.8 C	0.7 C	0.8 C
2	0.2 C	0.6 C	0.9 C	0.9 C
3	-0.6 C	-1.9 C	-3.2 C	—
4	0.7 C	1.5 C	1.7 C	1.7 C
5	0.5 C	1.3 C	1.4 C	1.4 C

This plasma pool affords an example of a protein solution from which the removal of pyrogenicity was effected by either of the agents alone.

Five hundred ml of this plasma were filtered through an S-6 pad, 40 sq inches per liter. After filtration a pyrogen test showed the following.

<i>Rabbit</i>	<i>1 hr</i>	<i>2 hr</i>	<i>3 hr</i>	<i>Max rise</i>
1	-0.6 C	-0.7 C	-0.5 C	—
2	-0.2 C	0.4 C	0.4 C	0.4 C
3	-0.2 C	0.4 C	0.4 C	0.4 C

Five hundred ml of the pyrogenic plasma were stirred with 15 g of "decalso" for a half-hour and were filtered through an S-3 pad, 40 sq inches per liter. The filtered material gave the following pyrogen test.

<i>Rabbit</i>	<i>1 hr</i>	<i>2 hr</i>	<i>3 hr</i>	<i>Max rise</i>
1	0.2 C	0.4 C	0.5 C	0.5 C
2	-0.3 C	-0.1 C	0.1 C	0.1 C
3	-0.3 C	0.0 C	-0.1 C	—

REMOVAL OF PYROGEN FROM A 25 PER CENT NORMAL HUMAN SERUM ALBUMIN SOLUTION

A 25 per cent solution of albumin gave the pyrogen tests below. Its pyrogenicity is better indicated by the fact that one of the rabbits died immediately after the test, and the other two were moribund, than by the actual temperature rises shown.

<i>Rabbit</i>	<i>1 hr</i>	<i>2 hr</i>	<i>3 hr</i>	<i>Max rise</i>
1	0.4 C	0.2 C	0.2 C	0.4 C
2	0.4 C	0.1 C	0.1 C	0.4 C*
3	1.2 C	1.5 C	1.9 C	1.9 C

* Rabbit died.

Earlier work has shown that with the highly concentrated albumin solutions the 30 g of "decalso" per liter usually used were ineffective even when followed by S-6 filtration. Likewise, the use of 60 g of "decalso" per liter of solution was only slightly effective. Five hundred ml of the foregoing solution were stirred one half-hour with 120 g of "decalso" (240 g per liter), and then were filtered through an S-6 pad, 40 sq inches per liter. The filtered albumin gave the following pyrogen test. None of the rabbits were ill.

<i>Rabbit</i>	<i>1 hr</i>	<i>2 hr</i>	<i>3 hr</i>	<i>Max rise</i>
1	-0.7 C	-0.5 C	-0.3 C	—
2	-0.2 C	-0.4 C	-0.4 C	—
3	-0.8 C	-0.4 C	-0.3 C	—

SUMMARY

The pyrogenicity of concentrated protein solutions can often be reduced by treatment with "decalso," and by filtration through S-6 pads of the Republic series.

The amount of "decalso" and the area of pad space used are functions of the degree of pyrogenicity and of the protein concentration of the solution, and for maximum effectiveness they should be determined for each solution to be examined. In practice, for 6 to 8 per cent protein solutions of mild pyrogenicity, 30 g of "decalso" and 40 sq inches of pad surface per liter of solution have given adequate reduction of pyrogenicity.

REFERENCES

- CO TUI, F. W., AND WRIGHT, A. M. 1942 The preparation of non-pyrogenic infusions and other intravenous fluids by adsorptive filtration. *Ann. Surg.*, **116**, 412-425.
- HOWARD, F., AND SPOONER, E. C. R. 1946 Removal of taints from water. *Chemistry and Industry*, 1946, 186-188.
- RANE, L., AND BALDWIN, D. M. 1945 Filtration of citrated plasma. *Science*, **101**, 494-496.
- TONNESEN, M., AND VESTERDAL, J. 1946 The pyrogenic effect of Danish penicillin. *Acta Pharm. Toxicol.*, **2**, 57-68.
- ZITTLE, C. A., DEVLIN, H. B., RODNEY, G., AND WELCKE, M. 1945 Removal of bacterial pyrogens from protein hydrolyzates. *J. Lab. Clin. Med.*, **30**, 75-79.