

Further Studies of Specific Precipitin Antiserums for the Protein of Cancer Tissue

II. The Application of *in Vivo* Absorption*

Lawrence S. Mann, M.D.,** and William H. Welker, Ph.D.

(From the Departments of Physiological Chemistry and Surgery, University of Illinois College of Medicine, Chicago, Ill.)

(Received for publication October 17, 1942)

When rabbits are injected with tissue proteins, precipitin antibodies for blood serum proteins appear in the serum in addition to precipitins for the tissue proteins (5, 6, 2, 1). There are three methods by which the former can be removed; namely, absorption *in vitro*, absorption *in vivo*, and allowing sufficient time to elapse following the injection for the exhaustion of blood proteins from the depots of tissue fixed on aluminum cream. Spinka and Weichselbaum (6) attempted to remove the blood serum protein precipitins from kidney and liver antiserums by absorption *in vitro*, using solutions of blood albumin and pseudoglobulin. They were able to rid the serum of the blood protein antibodies, but the tissue protein antibodies were also removed partially or entirely and invariably the potency of the tissue protein precipitins was definitely reduced. The absorption *in vitro* method failed to give us satisfactory results in similar experiments with cancer tissue. Hektoen and Welker (4) observed that the negative phase in precipitin production was specific. In rabbits immunized to many antigens, the injection of a single antigen resulted in the disappearance of the antibody for that antigen from the blood stream, with little or no effect on the titers of the rest of the antibodies. Spinka and Weichselbaum (6) were unsuccessful with the *in vivo* absorption technic in their work on precipitins for liver and kidney proteins. These investigators found that if sufficient time is permitted to elapse after the injection of tissue fixed on aluminum cream, the blood protein precipitins begin to drop in titer and finally disappear altogether. The presumption is that the blood proteins, being present in much smaller amounts than the tissue proteins, will disappear from the depots long before the tissue antigens become exhausted. This observation had been made previously by Hektoen and Welker (3) in their work on multiple antigens fixed on aluminum cream. When the serums of these animals no

longer react with blood proteins, autolysates of the tissue can be used to determine whether the serum contains any precipitins for the protein of the tissue. By this method they were able to prepare specific antiserums for liver and kidney proteins. It seems desirable to reinvestigate the possible use of the *in vivo* absorption method in connection with the use of tissue antigens. If this method could be successfully applied in the preparation of serum without affecting the titer of the tissue protein precipitins many months could be saved in their preparation.

EXPERIMENTAL

Rabbits were injected with tissue from carcinoma of the human breast, colon, kidney, and stomach, and with normal human kidney fixed on aluminum cream. Two to three weeks after injection the animals were bled by cardiac puncture and the serums tested for antibodies for blood serum proteins. On the following day the animals that reacted with both albumin and pseudoglobulin were injected by way of the marginal ear vein with 5 cc. of a 1 per cent solution of each of these proteins in physiological salt solution. If they had antibodies to only pseudoglobulin, 5 cc. of a 1 per cent solution of this protein was injected. Blood samples were taken at 1, 2, and 24 hour intervals. The serums of the 1 and 2 hour samples were titrated against the blood proteins and also against autolysates (5) of tissue proteins. The 24 hour sample was titrated against blood serum proteins. The results on 10 animals are shown in Table I.

DISCUSSION

Antibodies to blood proteins could not be detected in the serums of 9 of the animals at the end of 1 and 2 hour intervals after intravenous injection of the proteins, but reappeared in 24 hours. In 5 of these animals antibodies for tissue proteins were present 1 and 2 hours after intravenous injection, whereas in 4 they could not be detected. In one animal it was

* This investigation was aided by a grant from Eli Lilly and Company.

** Research Fellow in Surgery.

impossible to remove the antibodies for blood serum proteins, even after a second injection of twice the quantities of albumin and pseudoglobulin.

While it is possible to apply the absorption *in vivo* method for the preparation of specific antiserums for

proteins. However, the percentage of serums obtained by the application of this method is relatively low.

2. This technic is valuable in that the time required to obtain a specific antiserum for tissue protein is reduced from several months to a few weeks.

TABLE I

Animal No.	Tissue antigen fixed on aluminum cream	Titer of serum against blood proteins before intravenous injections		Protein solution injected intravenously	Titer of serum against blood protein after <i>in vivo</i> absorption						Reaction of serum with respective autolysates after intravenous injection	
		Albumin	Pseudo-globulin		1 hour		2 hours		24 hours		1 hour	2 hours
					A	P	A	P	A	P		
329	Cancer of breast, No. 9	0	4	5 cc. 1% P	0	0	0	0	0	4	++	++
348	Cancer of kidney, No. 101	0	4	5 cc. 1% P	0	0	0	0	0	4	++++	++++
551	Cancer of breast, No. 111	0	1	5 cc. 1% P	0	0	0	0	Animal died		0	0
566	Cancer of breast, No. 111	0	4	5 cc. 1% P	0	0	0	0	0	4	0	0
570	Cancer of breast, No. 111	0	4	5 cc. 1% P	0	0	0	0	Animal died		0	0
572	Cancer of breast, No. 111	0	4	5 cc. 1% P	0	0	0	0	0	4	0	0
983*	Normal human kidney	2	4	5 cc. 1% A 5 cc. 1% P	0	0	0	0	1	3	++++	++++
982	Normal human kidney	3	3	5 cc. 1% A 5 cc. 1% P	0	0	0	0	3	3	++++	++++
785	Cancer of stomach, No. 103	3	4	5 cc. 1% A 5 cc. 1% P	0	0	0	0	3	3	++	++
994	Cancer of colon, No. 102	3	4	5 + 10 cc. 1% A 5 + 10 cc. 1% P	3	3	3	3	3	3	?	?

Precipitin reaction to blood proteins:

- A = Albumin.
- P = Pseudoglobulin.
- 0 = No reaction.
- 1 = 1:100.
- 2 = 1:1,000.
- 3 = 1:10,000.
- 4 = 1:100,000.

* Experiment duplicated once in same animal.

Reaction of antiserums with autolysates:

- ++ = Reacted with 3 to 5 samples of autolysates.
- ++++ = Reacted with 8 to 10 samples of autolysates.

cancer tissue with satisfactory results, the percentage of successful experiments is low. Many of the rabbits die as a result of intravenous injection of the antigens used for the removal of their antibodies, and in some we were unable to remove the antibodies for blood proteins by this method. Finally, the antibodies for tissue proteins may be absorbed as well as those for blood serum protein. In spite of all these difficulties, however, our results show that it is possible to prepare specific antiserums for tissue proteins in the course of a few weeks, as against 6 months or longer, by allowing sufficient time to elapse for the exhaustion of the blood proteins from the aluminum cream depots.

SUMMARY AND CONCLUSIONS

1. By the use of the absorption method *in vivo* we have been able to produce specific antiserums for tissue

REFERENCES

1. BUCHHOLZ, A. M. Study of the Effect of Specific Kidney Antisera on the Normal Kidney. Proc. Soc. Exper. Biol. & Med., **43**:20-22. 1940.
2. COHEN, H. R., and FRED, V. C. Preparation and Diagnostic Value of an Antiserum for Placental Protein. Proc. Soc. Exper. Biol. & Med., **43**:22-23. 1940.
3. HEKTOEN, L., and WELKER, W. H. Precipitin Production in Rabbits Following Intramuscular Injection of Antigen Adsorbed by Aluminum Hydroxide. J. Infect. Dis., **53**:309-311. 1933.
4. HEKTOEN, L., and WELKER, W. H. The Specificness of the Negative Phase in Precipitin Production. J. Infect. Dis., **57**:337-344. 1935.
5. MANN, L. S., and WELKER, W. H. A Specific Precipitin Antiserum for Carcinoma Protein. Am. J. Cancer, **39**:360-364. 1940.
6. SPINKA, I., and WEICHSELBAUM, P. K. Antisera for Organ-Proteins. Proc. Soc. Exper. Biol. & Med., **38**:447-449. 1938.