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Immunologic Studies in Thermal Injury: Heterophile Antibodies.* (32033)

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A previous study(1) performed on rats with experimentally induced skin burns, demonstrated formation of agglutinins directed against syngeneic erythrocytes. These hemagglutinins could be demonstrated in the thoracic duct lymph but not in blood serum. Erythrocytes of some animals gave a positive direct Coombs test. The serological findings were interpreted by postulating that the burned animals form auto-hemagglutinins which are removed by red blood cells from the blood circulation but not from the lymphatic circulation.

Attempts were also made to demonstrate formation of heterophile antibodies directed against erythrocytes of foreign species. However, such antibodies were not found in burned rats. In considering the possibility that another species may be a better subject for studies on heterophile antibodies, the present experiments were performed on guinea pigs. In addition to the search for hetero-hemagglutinins, sera of burned guinea pigs were studied for the presence of anti-gamma globulin factors resembling the rheumatoid factor. These studies were later extended to rabbits, and a few sera from human patients with severe burns were also examined.

Materials and methods. Sera. Non-inbred

male albino guinea pigs of the Hartley strain, weighing 300 to 350 g, were purchased from Stuart Banta, Hagan, N. Y. Thirty-nine guinea pigs were subjected to full-thickness skin burns by a procedure described previously(1,2). A thermally-regulated metal surface maintained at 250°C was applied to 20% of the body surface area of the animals under ether anesthesia. After the first burn, 27 animals received 5 additional skin burns on a 1 sq cm area at weekly intervals. The repeatedly burned guinea pigs were exsanguinated by cardiac puncture one week after the last injury. The remaining 12 animals were exsanguinated at different times from 1 to 56 days after their first and only burn.

Sequential serum samples were obtained from 4 rabbits which received 3 skin burns in a manner similar to that used for guinea pigs. The rabbits were bled before injury and one week after the first, second, and third burning.

Human sera were obtained from 5 patients suffering from severe skin burns, hospitalized at the New York University Medical Center.

Erythrocytes. Human and animal blood was drawn into ACD solution and preserved for not longer than one week at 4°C. Before use, the erythrocytes were washed 3 times with phosphate-buffered saline solution, pH 7.2.

Serologic tests. Hemagglutination tests were performed by a procedure described previously(1). For detection of anti-human gamma globulin factors, the latex slide test (3), and a hemagglutination test with sensitized human red blood cells (SHC)(4), were

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TABLE I. Anti-Rat Hemagglutinins in Sera of Burned Guinea Pigs.

Sera obtained from:	Number of sera with titer of							Total
	≤8	16	32	64	128	256	≥512	
I. Guinea pigs burned 6 times	4	1	0	4	8	6	4	27
II. Group I before injury	15	1	0	0	0	0	0	16
III. Guinea pigs burned once	4	5	2	1	0	0	0	12
IV. Normal guinea pigs	7	3	2	0	0	0	0	12

TABLE II. Hemagglutination Tests with Sensitized Human Erythrocytes and Sera of Burned Guinea Pigs.

Sera obtained from:	Number of sera with a titer of						Total
	≤2	4	8	16	32	≥64	
I. Guinea pigs burned 6 times	13	4	5	3	1	1	27
II. Group I before injury	16	0	0	0	0	0	16
III. Guinea pigs burned once	12	0	0	0	0	0	12
IV. Normal guinea pigs	11	1	0	0	0	0	12

employed. The latex test was performed with RA reagent of Hyland Laboratories, Los Angeles, Calif. For the SHC test, human erythrocytes were sensitized by an incomplete anti-Rh (anti-CD) serum "Ri." Serum "Ri" was kindly supplied by Dr. Marion Waller of the Medical College of Virginia, Richmond. The procedure used has been described in detail(5).

Treatment with sulfhydryl reagent(6). Selected serum samples from repeatedly burned guinea pigs were diluted 1:4 and incubated with an equal volume of 0.2 M 2-mercaptoethanol for 1 hour at 37°C. Such samples were then immediately tested by the hemagglutination and SHC tests.

Results. Sera obtained from burned guinea pigs, as well as control sera from normal guinea pigs, were tested against erythrocytes of various species including man, ox, sheep, pig, rabbit, guinea pig, rat, mouse, and chicken. In these screening tests, it was noted that sera obtained from burned guinea pigs gave a high titer of agglutination with rat erythrocytes but not with erythrocytes from any of the other species tested. Titration experiments were then carried out with rat erythrocytes. Results of these experiments are summarized in Table I. It may be noted that 22 of 27 repeatedly burned guinea pigs developed anti-rat hemagglutinins with titers of 64 or more. No significant differences in the hemagglutinating titers of the sera were observed by using erythrocytes of 4 different rat strains: Wistar,

Fisher, Lewis, and Sprague-Dawley. In contrast, normal guinea pig sera, including sera from the burned guinea pigs obtained before injury, generally exhibited anti-rat erythrocyte titers of 16 or less. Sera from animals exsanguinated at various times after the first burn did not show titers of anti-rat hemagglutinins as high as those obtained with sera from the repeatedly burned animals.

The serum samples were studied by the latex test for the presence of anti-human gamma globulin factors. Most sera obtained from repeatedly burned animals gave positive latex tests. All serum samples were then tested by the SHC test after absorption of the sera with human erythrocytes (Table II). Definite positive reactions (a titer of 8 or more) were obtained with sera of 10 of the 27 repeatedly burned guinea pigs. None of the other serum samples gave a significantly positive reaction.

In Table III, results of an experiment are presented for which a serum was selected

TABLE III. Effect of Heat and 2-Mercaptoethanol (2-ME) on Serological Activity of Serum from a Repeatedly Burned Guinea Pig.

Type of serum tested	Agglutination titer with	
	Rat erythrocytes	Sensitized human erythrocytes
Untreated	64	16
Heated for 30 min at		
56°C	64	16
63°C	<4	16
70°C	<4	<4
Treated with 2-ME	<8	16

TABLE IV. Hemagglutination Tests with Sera from 4 Repeatedly Burned Rabbits.

Serum samples obtained	Agglutination titer with	
	Rat erythrocytes	Sensitized human erythrocytes
Before injury	<8, 8, <8, <8	<2, <2, <2, <2
After first burn	16, 64, 64, 16	4, 2, <2, <2
After second burn	16, 64, 128, 16	4, 8, 2, <2
After third burn	16, 64, 128, 64	4, 32, 64, <2

that contained both anti-rat hemagglutinin and anti-human gamma globulin factor. It may be noted that the hemagglutinin was destroyed by heating at 63°C and by 2-mercaptoethanol, whereas the anti-human gamma globulin factor resisted these treatments. Analogous results were obtained in studying 3 other similar sera.

Sequential serum samples obtained from 4 rabbits with thermal injury were studied by the rat cell hemagglutination and SHC tests (Table IV). The experimental materials were not sufficient for precise evaluation. However, there appeared to be very little doubt that at least some rabbits formed anti-rat hemagglutinins and anti-human gamma globulin factors.

Finally, sera from 5 patients with severe skin burns were also examined. As may be noted in Table V, sera of 3 patients contained rather potent anti-rat hemagglutinins and sera of 2 patients gave definite latex tests.

Discussion. Heterophile antibodies have attracted considerable attention in our laboratories (7-9). Heterophile antibodies should be defined as antibodies combining with a quite different antigenic material than the one used for immunization. Forssman antibodies are classical examples of heterophile antibodies. The unexpected cross reactions given

by Forssman antibodies were satisfactorily explained by demonstration that various, even biologically far-remote species, carry similar antigenic determinants in their tissues.

The term "heterophile antibodies" has been also frequently used in relation to antibodies detectable in serodiagnostic tests employing antigens other than those derived from causative agents responsible for a given disease. Antibodies in infectious mononucleosis sera which combine with sheep and bovine erythrocytes (10) are frequently referred to as heterophile antibodies. The explanation most frequently offered for the appearance of heterophile antibodies such as those encountered in infectious mononucleosis assumes that the causative agents (in this case a virus) share antigenic determinants with the antigen used for detection of antibodies (in this case sheep or bovine erythrocytes). Alternatively, it has been postulated that a morbid process may result in alterations of autologous antigens to the point where they resemble foreign antigens. These altered antigens would stimulate formation of antibodies which would combine with foreign antigens. The latter hypothesis was substantiated by studies on the nature of the rheumatoid factor. It was shown in previous studies (11,12) that rabbits injected with denatured autologous gamma globulin form antibodies combining with foreign gamma globulin, first of all, human gamma globulin. This finding was explained by postulating that the denaturation brings about conformational changes of the autologous gamma globulin resulting in the appearance of novel antigenic determinants which resemble those naturally occurring in a foreign gamma globulin.

TABLE V. Rat Cell Hemagglutination and Latex Slide Tests with Sera of 5 Patients Suffering from Severe Skin Burns.

Initials	Age in yr	Sex	% of total body surface exposed to burn	Outcome	Serum samples obtained X days after injury	Agglutination titer with	
						Rat erythrocytes	RA latex
A.M.	27	♂	45	Recovery	141	256	<8
C.T.	28	♂	65	Death after 28 days	21, 28	16	<8
S.B.	61	♀	30	Recovery	27	<8	<8
J.H.	54	♀	22	Death after 13 days	7	256	256
W.B.	28	♂	38	Death after 30 days	7, 9	128	1024

It would appear that the anti-rat hemagglutinins detectable in sera of burned guinea pigs, rabbits and humans were elicited by the autologous tissue antigens altered as a result of the injury. An alternative possibility should also be considered that these antibodies were engendered by bacterial infection complicating the wound healing. This interpretation, however, requires a rather unlikely assumption that all the animals suffered from an infection by microorganisms sharing antigens with rat erythrocytes. The final answer to this point was sought in experiments on germ-free animals. Thus far, however, the burned germ-free animals could not be maintained alive long enough to conclude the experiments. Formation of anti-gamma globulin factors might have been stimulated by denatured autologous gamma globulin in a similar fashion as was the case in the above quoted studies(11,12).

Summary. Anti-rat hemagglutinins and anti-human gamma globulin factors were demonstrated in sera of guinea pigs and rabbits exposed to repeated thermal injury. Similar factors were also found in sera of

some patients with severe skin burns.

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Effect of Some Analogues of Bradykinin Upon Vascular Permeability.* (32034)

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Increased vascular permeability is probably an important and early response of the microcirculation to injury in an inflammatory response(1). Bradykinin has been shown to induce several changes associated with experimental inflammatory responses including vasodilatation(2-4), increased vascular permeability(5), and margination of leukocytes

(6). It was therefore of interest to assess some of the synthetic analogues of bradykinin in regard to their effect upon vascular permeability, and to compare this action with some other pharmacologic properties of these compounds.

Materials and methods. The synthetic bradykinin and analogues listed in Table I were synthesized by Nicolaides and his associates(7). Permeability properties were measured in the skin of living, unanaesthetized guinea pigs by observing changes in permeability induced by intradermal injection of these compounds suitably diluted in buffer. This technique has been described by Miles

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