TABLE I.
Excitability of Rectus Muscle Increased by Eserine, Acetone, and Methyl Alcohol.

		Rheobase				
		Before treatment		After treatment		
Treatment	No. of observations	Range, v.	Avg, v.	Range, v.	Avg, v.	Difference, v.
Eserine	10	.58- .71	.669 ± .014	.34- .58	.485 ± .024	.184 ± .028
Acetone	10	.65- .78	.717 ± .014	.46- .66	.583 ± .020	.134 ± .024
Methyl-alcohol	10	.65- .77	.705 ± .012	.44- .66	.569 ± .019	.136 ± .022
Ringer's solution (Blank control)	10	.92-1.34	1.106 ± .041	.90-1.33	1.085 ± .044	.021 ± .060

		Chronaxie				
		Before treatment		After treatment		
Treatment	No. of observations	Range, msec.	Avg, msec.	Range, msec.	Avg, msec.	Difference, msec.
Eserine	10	1.30-7.46	3.649 ± .563	1.11- 8.14	4.007 ± .782	.358 ± .964
Acetone	10	.40-8.55	3.086 ± .710	1.54-11.0	3.859 ± .830	.773 ± 1.092
Methyl-alcohol	10	.77-6.22	4.104 ± .535	1.72- 6.75	4.810 ± .532	.706 ± .754
Ringer's solution (Blank control)	10	.87-7.96	4.064 ± .788	1.91- 9.64	5.308 ± .877	1.244 ± 1.187

It was found that rheobase was uniformly decreased by all these agents (Table I), indicating that the excitability of the muscle was definitely increased according to Chao.[1] Similar treatment with Ringer's solution gave no significant change.

The chronaxie showed insignificant change, and we agree with Chao[1] that it is not a valid measure of excitability in such a case.

Summary. With the threshold intensity as a criterion, the excitability of the rectus muscle is definitely increased by eserine, acetone and methyl-alcohol.

1 Chao, I., *The Science Reports of National Tsing Hua Univ. Series B*, **2**, 183.

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Detection of Acrolein by Qualitative Immunochemical Analysis. (17318)

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In a previous report[1] it was shown that acrolein can produce a condition in experimental animals similar in its clinical and pathologic manifestations to that accepted as "shock". The problem of the detection of acrolein in blood or tissue by some specific method next presented itself. Acrolein is found to be a chemically active and highly unstable compound that does not retain its characteristics for any length of time. These two properties alone would make it difficult to detect by the usual qualitative methods of organic analysis, especially when acrolein is present in small quantities. Secondly, as will be shown, there is experimental evidence indicating that acrolein combines directly with protein, and no longer exists in a free state. It thus becomes obvious that the usual qualitative chemical methods of detection cannot be used.

Another possible method for the detection was suggested by a postulated chemical com-

1 Kamen, G. F., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 363.

bination of acrolein with protein. If such a reaction takes place in the body, then it should follow that the configuration and chemical properties of the native protein molecule would be changed. Theoretically, protein molecules changed in this manner behave as substances foreign to the body and may act in the capacity of an antigen and cause antibody formation. Applying this principle to the pathologic physiology of burns and burn shock, one might assume that any acrolein that may be produced as a result of a burn probably combines with body protein locally or systemically. In the combined state it theoretically might be detected. Animal experimental studies were undertaken to demonstrate, if possible, by indirect evidence chemical changes in protein molecules treated with acrolein, in order to test the validity of these considerations.

Laboratory procedure. Freshly distilled acrolein was added to normal human and rabbit sera, in sufficient quantity so that the mixtures contained 0.5 and 1.0% of acrolein by volume. These mixtures were immediately diluted 1:5 with physiological salt solution. Sheep serum was prepared in the same manner to contain 1.5% of acrolein. The flasks were stoppered with cotton; all were then placed in a constant temperature water bath at 37°C, some for 4 days and others for 10 days to permit any excess acrolein to volatilize. At the end of this time, the contents were transferred to diaphragm rubber-stoppered bottles that were kept at refrigerator temperature (5°C). The acroleinized serum incubated at 37°C for 10 days undergoes further changes after approximately one month and will give nonspecific precipitin reactions when mixed with normal horse, rabbit, sheep or human serum. The acroleinized serum incubated at 37°C for 4 days and kept for as long as nine months was found to be free from nonspecific reactions. The microprecipitin test was used; the reactions were read within 5 minutes at room temperature. For the possible demonstration of cross reactions with formolized serum, normal human and rabbit sera were each prepared to contain 0.8% formalin gas and placed in a constant temperature water bath at 37°C for 10 days with cotton stoppers to allow any excess formalin to volatilize. On the tenth day the formalized serum was removed and diluted 1:5 with physiologic saline solution and transferred to diaphragm rubber-stopped bottles and stored at room temperature.

Immunization. Male rabbits, approximately 7 lb in weight and 5 to 6 months of age, were used. All injections were given intravenously. Acroleinized human serum incubated for 10 days was injected daily for an average of 6 days. After this a rest period of a week was allowed. The injections were repeated in this manner for a series of 4 courses of injections. The daily amount given in the first series was 0.5 ml, 1.0 ml, in the second, 2.0 ml, in the third and the final series of injections 3.0 ml. Acroleinized rabbit serum incubated for 10 days was injected in a like manner except, the first course of injections was 1.0 ml daily of 1:10 dilution; in the next 5 courses of injections a 1:5 dilution was used. The amount injected daily in the second series of injections was 1.0 ml, third 2.0 ml, fourth 3.0 ml, fifth 3.0 ml. In the sixth and final series 5.0 ml were injected every other day of the 6 day period.

Eight days after the completion of the series of injections, 3.0 ml of blood were collected from the ear vein of each of the animals receiving acroleinized serum incubated for 10 days and the antibody titer determined. Later the same day, 40.0 ml of blood were removed from the heart of each animal. To determine if bleeding would influence the antibody titer, an additional series of injections of acroleinized serum after the third series was then given as outlined above. Antibody titer increases were only slight.

Acroleinized human or rabbit serum incubated for 4 days was injected in daily single doses of 1.0 ml initially, 2.0 ml for the second, 3.0 ml for the third, and 3.0 ml for the fourth and final series of injections. Ten days following the last injection each animal was bled from the heart of approximately 50 ml.

Formalized human or rabbit serum was injected daily for 6 days and then a rest period of about a week was allowed. This was repeated for 5 courses of injections. The amount given initially at each injection was 1.0 ml,

TABLE I.

Antiserum	Test antigen	Antigen dilution 1:5	1:25	1:125	1:625	1:3125	Titers
Anti-acroleinized human serum	Acroleinized human serum	++++	++++	++++	+	—	1:125
	Normal human serum	++++	++++	++++	++	—	1:625
	Acroleinized rabbit serum	++++	++++	++	—	—	1:125
	Normal rabbit serum	—	—	—	—	—	0
	Acroleinized sheep serum	++++	++++	+++	+	—	1:125
	Normal sheep serum	—	—	—	—	—	0
	Acrolein	—	—	—	—	—	0
Anti-acroleinized rabbit serum*		—					

* No precipitins demonstrated against any of the test antigens including acroleinized rabbit serum.

1.0 ml for the second and third, 2.0 ml for the fourth and 3.0 ml for the final series of injections. A preliminary bleeding one week after the third series of injections showed, in those rabbits receiving human serum, precipitins that reacted against normal human serum and formalized human serum, but not against normal or formalized rabbit serum. No precipitins could be demonstrated in the blood of those rabbits that received rabbit serum. The formalin concentration was then adjusted from 0.8% to 2.0% (formalin gas) and incubated at 37°C for 24 hours in sealed flasks. Two additional series of injections were then given and the rabbits bled from the heart 9 days after the last injection. The precipitin reactions were the same as before.

Results. Results are summarized in the table. While the rabbits injected with acroleinized rabbit serum did not respond, those injected with acroleinized human serum did produce precipitins. These precipitins reacted not only with acroleinized human serum but also with acroleinized rabbit and acroleinized sheep serum. As expected, they also reacted with normal human serum due to the formation of precipitins for normal human serum protein. That the cross reactions with acroleinized rabbit and sheep sera were due to an antibody directed against acrolein in a combined form with protein itself is indicated by the observation that the antiserum did not react with untreated rabbit or sheep serum and also did not react with pure acrolein.

Discussion. The molecular configuration of rabbit serum protein is altered by acrolein but not sufficiently to act in the capacity of an antigen and cause antibody formation when injected into rabbits. Apparently the basic molecular structure of the protein characteristic of the human and rabbit species is not altered by acrolein. This is shown by the failure to form antibodies when rabbits are injected with acroleinized rabbit serum, and also by the response of the rabbits injected with acroleinized human serum by producing antibodies that reacted not only against acroleinized human serum but with also normal human serum proteins. Slight changes in the configuration of the protein molecules, however, did occur. These changes remained the same regardless of the basic molecular structure of the protein as evidenced by cross reactions of antiacroleinized human serum with acroleinized serum of other species. That the antibody was directed against the acrolein in a combined form with protein itself is indicated by the observation that the antiserum did not react with untreated rabbit or sheep serum and also did not react with pure acrolein. It would seem, therefore, that foreign protein conjugates of acrolein are necessary to produce circulating antibodies that can be detected. From the chemical nature of acrolein and protein, it may be that this linkage takes place through indole nitrogen and amino

groups.

Conclusion. 1. These studies indicate that acrolein combines directly with some portion of the protein molecule to produce a conjugate that can be employed in the specific detection of acrolein by qualitative immunochemical methods.

2. The basic molecular pattern of human and rabbit serum protein is not changed when combined with acrolein at 37°C for 4 days. The addition of molecules of acrolein, however, does change the configuration of the protein molecule without loss of species characteristics. This is shown by the failure to form antibodies when rabbits are injected with acroleinized rabbit serum, and also by the response of the rabbits injected with acroleinized human serum by producing antibodies that reacted not only against acroleinized human serum but normal human serum as well. These changes in the molecular configuration were specific for acrolein regardless of the basic molecular pattern used, as evidenced by cross reactions of antiacroleinized human serum with acroleinized serum of other species.

3. Foreign protein conjugates of acrolein are necessary to produce circulating antibodies that can be detected in a test tube.

Studies are now in progress on the detection of acrolein in the blood and tissues of individuals who have suffered burns.

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Inhibitory Effect of Nitrogen Mustard (Bis Beta-Chloroethyl Amine) on Lesions of Experimental Serum Hypersensitiveness.* (17319)

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Experimental serum hypersensitiveness has been studied intensively because it may serve as a useful model in promoting understanding of certain diffuse vascular diseases appearing in man. The earlier investigations of experimental hypersensitiveness were concerned primarily with morphologic characteristics of the lesions and their similarity to those of the human diseases. More recent investigations, concerned with pathogenesis, have dealt with the relationship of immunologic and vascular changes.[1-4] The information obtained has led to the general conclusion that the development of humoral antibody and cutaneous hypersensitiveness is apparently related to the development of the vascular lesions. The role of immunological factors has been further investigated by determining the incidence of vascular lesions in serum-injected animals treated with drugs which may 1) prevent antigen-antibody combination or the vascular response to such combination or 2) inhibit antibody formation. Salicylates and dicumarol inhibit antigen-antibody combination *in vitro*,[5,6] but as yet there is no

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