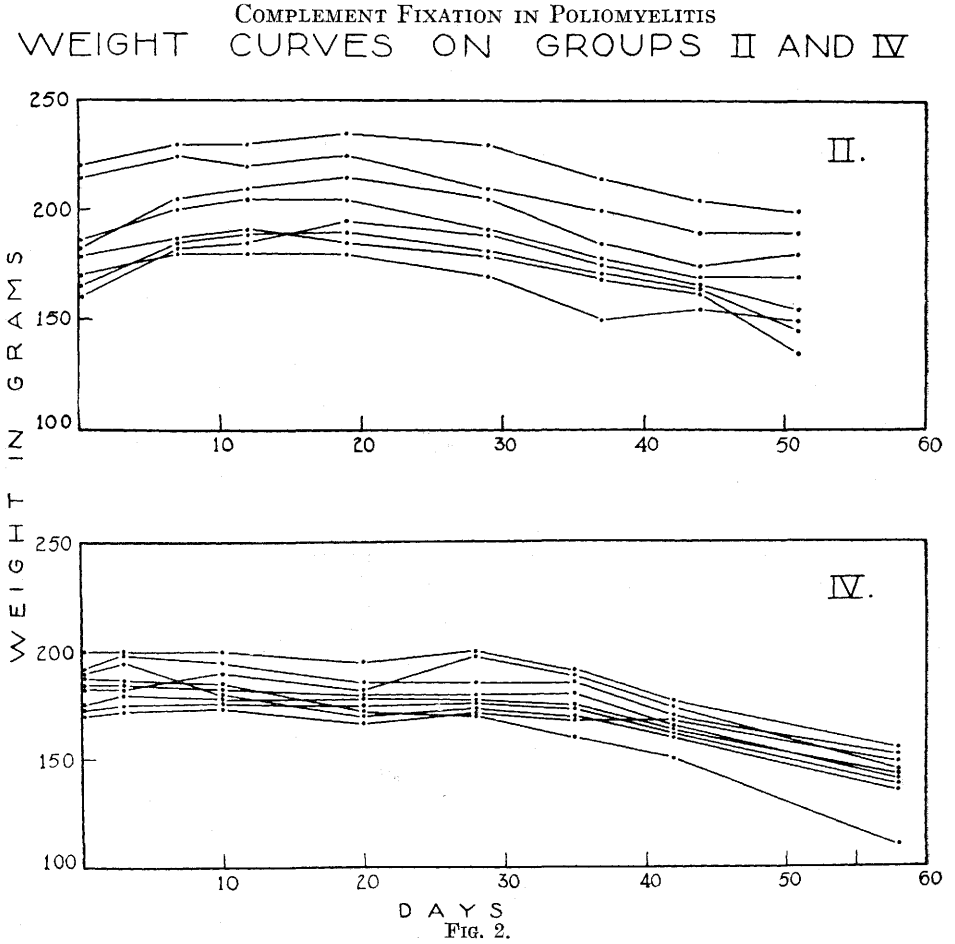

FIG. 2.

which permits accumulation of fat within the liver cells, and that this effect operates separately from that of extrinsic deficiency of lipotropic factors.

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Complement-Fixation in Experimental and Human Poliomyelitis.*

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Although many attempts have been made to find a simple immunological means of studying poliomyelitis, only the neutralization reaction has yielded consistent and reliable results. Possible reasons for the lack of success of precipitin and complement-fixation tests have been discussed by several authors.[1] The possibilities include (a) low reactivity or avidity of the neutralizing antibodies, (b) small size of the virus particle, and (c) insufficient concentration of virus in the antigen

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

1 Schultz, E. W., Gebhardt, L. P., and Bullock, L. T., *J. Immunol.*, 1931, **21**, 171; Raffel S., and Schultz, E. W., *J. Immunol.*, 1940, **39**, 256.

TABLE I.
Complement-fixation with Concentrated Lansing Virus and Sera from Immune Rats.*

Immunization history	Rat No.	Serum 1: 0	2	4	8	Serum control	Titer (units per ml)
Group a, vaccinated rats which developed "polio" on challenge, but survived with residual paralysis.	66	4	4	4	3	0	80
	67	4	2	±	±	0	20
	94	4	4	4	2	0	80
Group b, vaccinated rats which proved resistant to subsequent challenge	90	2	2	±	±	0	20
	91	1	1		0	0	+
	92	±	0	0	0	0	0
Group c, same as group b, vaccinated with a different preparation of formalized virus	63		4	4	4	0	>80
	64	4	4	4	±	0	>40
	95		4	2	4	0	>80
	100			4	4	0	>80
	102	4	4	4	4	0	>80
	107		4	4	4	0	>80
	113	4	4	4	4	0	>80
	116	4	4	4	4	0	>80

Groups a and c. Injected with 10^{-5} g formalized virus nitrogen, Preparation 91-2-4,[3] as follows: 2.5 x 10^{-6} g intradermally on 12/16/46, 5 x 10^{-6} g intramuscularly on 12/23/46, and 2.5 x 10^{-6} g intradermally on 12/30/46. Challenged on 1/6/47. Bled 1/21/47.

Group b. Injected with 3 x 10^{-6} g formalized virus nitrogen, Preparation 87[3] as follows: 2 x 10^{-3} g on 8/15/46 and 10^{-6} g on 8/20/46 intraperitoneally. Challenged 8/24/46. Rechallenged 9/27/46. Reinjected with 0.5 cc of 20% crude virus suspension twice weekly from 10/12/46 to 1/2/47. Bled 1/21/47.

* Virus preparation 91-2-4[3] at 2 x 10^{-5} g nitrogen per ml used as antigen; antigen control with one unit complement = 0, with ½ unit complement = 2, and with no complement = 4.

preparations used.

Recent work on the concentration of the Lansing strain of poliomyelitis virus[2] has provided more concentrated virus preparations than those previously employed in immunological tests. In a previous paper[3] the immunization of cotton rats with formalized preparations of highly concentrated Lansing virus and their protection against the intracerebral injection of large doses of active virus was reported. If the high degree of immunity produced in many of these animals was due to a high titer of humoral antibodies, it seemed possible by the use of concentrated virus as antigen that positive precipitin or complement-fixation tests could be demonstrated in such sera. Precipitin tests were attempted but proved negative. Complement-fixation tests, on the contrary, proved definitely positive.

The results obtained with sera from 2 groups of rats that had been injected with formalized virus and had proved refractory to relatively large amounts of active virus, as well as with sera from 3 immunized rats which developed poliomyelitis on subsequent challenge, are given in Table I. The complement-fixation tests were made as follows: The immune sera were incubated at 56°C for 20 minutes to destroy the complement present and mixed with saline to give the dilutions used. A 0.1 ml volume of each dilution was then distributed in small test tubes, to which was added 0.2 ml of a dilution of guinea pig complement containing 2 units, 0.6 ml of saline, and 0.1 ml of a 2-cycle virus preparation containing 10^{-5} g nitrogen per ml. Preliminary tests showed this concentration of virus to be devoid of anticomplementary properties when tested with fractional units of complement. Controls without antigen were set up for the entire range of dilutions of the sera. Controls for possible anticomplementary action of the antigen were also set up along with each set of tests. The tubes were shaken and incubated at 4°C overnight. To a 4% suspension of sheep red blood cells was added an equal volume of solution containing eight units of hemolysin per ml and 0.5 ml of this suspension of sensitized cells was added to each test tube. The tubes were shaken, in-

[2] Loring, H. S., and Schwerdt, C. E., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 291.

[3] Loring, H. S., Schwerdt, C. E., Lawrence, N., and Anderson, J. C., *Science*, 1947, **106**, 104.

cubated at 37°C for one hour, set in the refrigerator for 2 hours or slightly more to permit settling of cells, and read. The last tube showing 50% lysis was taken to determine the titer. Titer is expressed in these experiments as the number of units of complement-fixing antibody contained in one ml of undiluted serum. It is worthy of emphasis that in carrying out these tests the following precautions were observed: (a) The tubes were incubated at low temperature for eighteen hours to assure maximum reaction and minimal deterioration of complement. (b) The antigen was tested for possible anticomplementary properties by incubation with fractional units of complement in advance of and simultaneously with each group of tests. (c) A control for anticomplementary properties of each serum was made throughout the dilution range simultaneously with the actual test.

The data presented in Table I show that complement-fixing antibodies against active virus were present to a variable degree after immunization with formaldehyde-inactivated virus. As both the method of vaccination and the elapsed time before blood was drawn varied in these experiments, it was reasonable to expect the type of variation found. When blood was drawn about three weeks after the last injection of vaccine in the animals which were refractory to infection, Group c, uniformly high titers of >80 were found. Blood from vaccinated animals which developed poliomyelitis but recovered with some residual signs of the disease, Group a, also showed similar high titers. Samples taken five months after immunization, however, (Group b) gave relatively low titers although these animals had been given several injections of crude 20% infected-brain extract a few weeks previously.

As the vaccine used for immunization in the above mentioned experiments had been obtained from a virus preparation that may have contained some normal rat tissue proteins, the positive results could be explained by immunization to the normal tissue components as well as by immunization to virus. In order to study this question, the complement-fixing

TABLE II. Complement-fixation of Immune Sera with Concentrated Virus and Normal Tissue Antigen.

Immunization history	Sera	Degree of complement-fixation at serum dilutions and with antigens indicated: Virus; serum 1: 0	2	4	8	16	32	64	128	Normal; serum 1: 0	2	4	8	16	32	64	128	Normal 1/10; serum 1: 0	2	4	8	16	32	64	128	Serum control 0	2	4	8	Titer for Virus	Titer for Normal	Titer for normal 1/10
Group a. Pooled sera from 6 vaccinated rats which proved resistant to subsequent challenge*	Fresh	4	4	4	4	4	4			2	3	3	2	0	0			1	1	1	±	0	0			±	±	±	±	>320	80	+
	Frozen 7 days	4	4	4	4	4	4	3	±	3	4	4	4	2	0	0	0	3	3	2	1	0	0	0	0	±	1	±	0	640	160	40
Group b. Four vaccinated rats which proved resistant to subsequent challenge†	Fresh	4	4	4	2	0	0			4	4	2	0	0	0											+	0	0		160	80	
		4	4	4	2	0	0			4	4	3	±	0	0											±	0	0		160	80	
		4	4	4	±	0	0			3	4	2	0	0	0											0	0	0		>80	80	
		4	4	4	4	2	0			4	4	4	2	0	0											0	0	0		320	160	

* Injected with 10^{-5} g formalized virus nitrogen. Preparation 91-2-4.3 as follows: 2.5×10^{-6} g intradermally on 1/24/47; 5×10^{-6} intramuscularly on 1/31/47 and 2.5×10^{-6} g intradermally on 2/7/47. Challenged on 2/17/47. Bled 3/10/47.

† Same group of rats as Group c, Table I. Bled 4/8/47.

Virus preparation 91-2-43 used at 10^{-5} g nitrogen per ml; preparation of normal tissue components used at 10^{-5} g nitrogen per ml3 and at 10^{-6} g nitrogen per ml as indicated.

titer of each serum was determined with a similarly concentrated preparation of normal cotton rat brain and spinal cord in comparison with that of concentrated virus.

A total of 257 g of brains and spinal cords from normal cotton rats were processed by the procedure used for the preparation of the virus concentrate.[2] After 2 ultracentrifugal cycles of purification, the high molecular weight sediments obtained contained 0.85 mg of nitrogen. The yield of nitrogen found in this experiment was relatively higher than those obtained from normal tissue after 3 or 4 cycles of purification as previously reported. This normal constituent was used in the complement-fixation tests at the same concentration as that used for the virus preparation, namely 10^{-5} g nitrogen per ml, and also at one-tenth this concentration.

The results of this experiment are summarized in Table II. It may be seen that a sample of pooled immune serum from 6 immunized and refractory rats fixed complement when either the concentrated virus or the preparation from normal rat tissue was used as antigen. In all the tests made, however, the titer for virus (160 to 1280) was significantly higher than that for the normal preparation at either the same concentration, (titers of 80 to 160) or at one-tenth the concentration (titers of + to 40). Because of the difference in titers with the 2 antigens, the results suggest that complement-fixation in the presence of virus was due primarily to the reaction of antibody with virus rather than to a reaction of antibody with a normal component present in the virus preparation. Another possible explanation for positive complement-fixation with the antigen from normal tissue, in addition to that mentioned above, is that poliomyelitis virus produced in cotton rats may be related antigenically to certain normal brain tissue proteins. The latter phenomenon would be similar to that observed with several infectious agents and certain of the normal antigens of the host.[4,5,6]

Experiments were conducted with sera from normal rats to determine if antibodies were present which would fix complement in the presence of either virus or the normal tissue preparation. These results are summarized in Table III. It may be seen that complement-fixation occurred with most of the sera tested with virus as antigen in animals either 5 or 8 weeks old. Less consistent results and in general lower titers were found with the "normal" antigen. Several instances are recorded where positive tests were found with virus, but no antibodies to the "normal" antigen could be detected. These results are similar to those found by Casals and Palacios[7] and by Kidd and Friedewald[8] with non-specific antibodies occurring in normal serum. In the latter instances it was reported that the non-specific antibody could be eliminated by heating the serum to a higher temperature than that usually employed to destroy complement. It was of interest to determine if the reaction with the virus preparation and the "normal" antibody could be similarly eliminated. Two normal sera which fixed complement in the presence of virus after they had been heated at 56°C for 20 min. were heated for an additional 20 min. at 60°C and again tested with the virus concentrate as antigen. In both cases negative tests were now found indicating that the normal antibody had been eliminated by this method.

The same procedure was next applied to the 5 immune sera shown in Table II which had fixed complement in the presence of either virus or the "normal" antigen. After they had been heated 20 min. at 60°C, they were again tested with virus and with the "normal" antigen. In 4 of the 6 samples the reaction with the "normal" antigen was eliminated whereas positive complement fixation (titer of 20 to 80) was still found with the virus concentrate.

A consideration of the results obtained in all the experiments mentioned seemed to justify the conclusion that complement fixa-

[4] Duran-Reynals, F., *Yale J. Biol. and Med.*, 1940, **13**, 61.

[5] Chambers, L. A., and Henle, W., *Am. J. Path.*, 1941, **17**, 442.

[6] Knight, C. A., *J. Exp. Med.*, 1946, **83**, 281.

[7] Casals, J., and Palacios, R., *J. Exp. Med.*, 1941, **74**, 409.

[8] Kidd, J. G., and Friedewald, W. F., *J. Exp. Med.*, 1942, **76**, 543.

TABLE III.
Complement-fixation of Normal Rat Serum with Virus and with "Normal" Antigen.

History of animals	Virus preparation	Serum dilution and degree of complement-fixation with antigen shown: Virus; Serum 1: 0	2	4	8	16	32	"Normal";* Serum 1: 0	2	4	8	16	32	Serum control 0	2	4	8	16	32	Titer for virus	Titer for "Normal"
Group a. 8 wks old, kept in animal room 3 weeks before blood samples taken	91-2-4	3	3	3	1			0	0	0	0			0	0	0	0			40	0
	"	3	3	3	1			0	0	0	0			0	0	0	0			40	0
	96-7-8	2	3	1	±	0	0	2	1	±	0	0	0	1	1	0	0	0	0	20	±
	"	2	4	4	±	0	0	1	2	±	0	0	0	0	0	0	0	0	0	40	20
Group b. 5 wks old, bled when received from dealer	"	2	3	2	0	0	0	2	3	±	0	0	0	0	0	0	0	0	0	40	20
	"	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0
	"	0	2	4	4	±	0	0	4	4	3	0	0	0	0	0	0	0	0	80	80
	"	3	4	4	3	0	0	3	4	4	±	0	0	2	1	0	0	0	0	80	>40
	"	4	4	4	3	0	0	3	4	3	0	0	0	0	0	0	0	0	0	80	>40
	"	4	3	2	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	40	+
	"	±	±	±	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	"	2	4	4	3	0	0	1	3	4	2	0	0	0	0	0	0	0	0	80	80
	"	4	4	4	3	±	0	3	4	3	±	0	0	0	0	0	0	0	0	80	>40
	"	2	4	4	3	±	0	0	1	2	±	0	0	0	0	0	0	0	0	80	40
Group c. Pooled sera from 3 rats 5 wks old	100	0	0	0	0	0								0	0	0	0	0		0	

* "Normal" antigen = preparation from normal brain and spinal cord. Virus preparations 96-7-8 and 100 were prepared as previously described[3] and were used at a concentration of 10^{-5} g nitrogen per ml.

TABLE IV.

Complement-fixation Tests on Human Convalescent Sera with Concentrated Lansing Virus and Normal Cotton Rat Tissue Antigen.

Name	Case history*	Date of blood sample	Antigen†	Serum dilution, 1: 0	2	4	8	16	32	64	Serum Control	Titer
M.K.	♀, 23 yr, A.P. 8-1-46	1-28	Virus				4	4	1	0	0	>160
		"	"		4	4	4	4	2		0	320
		"	Normal		0	0	0	0	0		0	0
		3- 5	Virus	4	4	4	4	4	3		0	320
		"	Normal	0	0	±	0	0	0		0	0
J.I.	♀, 10 mo., A.P. 7-20-46	1-28	Virus	2	4	4	4	2	0		0	160
		"	Normal	0	0	0	0	0	0		0	0
		3-5	Virus	1	3	3	1	0	0		0	40
		"	Normal	0	0	0	0	0	0		0	0
J.M.	♂, 28 yr, A.P. 10-11-46	1-28	Virus	2	4	4	2	±	0		0	80
		"	Normal	0	0	0	0	0	0		0	0
		3- 5	Virus	1	2	2	1	0	0		0	40
		"	Normal	0	0	0	0	0	0		0	0
R.He.	♂, 33 yr, A.P. 9-7-46	1-28	Virus	1	1	1	1	1	±		0	+
R.H.	♂, A.P. 8-46	"	"	1	1	1	1	±	±		0	+
S.McI.	♀, 4 yr, A.P. 8-13-46	"	"	±	±	±	±	±	±		0	0
N.K.	♀, 28 yr, A.P. 8-16-46	"	"	0	0	0	0	0	0		0	0
F.O'C.	20 yr, A.P. 9-16-46	"	"	0	0	0	0	0	0		0	0
N.W.	♂, 21 yr, A.P. 6-5-46	"	"	0	0	0	0	0	0		0	0
D.W.	♀, 4 yr, bulbo-spinal poliomyelitis, 10-1-46	"	"	0	0	0	0	0	0		0	0
F.C.	♂, 10 yr, A.P. 7-15-46	"	"	0	0	0	0	0	0		0	0
G.J.	♂, 26 yr, A.P. 7-15-46	"	"	±	±	±	±	±	±		0	0
G.H.	♂, 6 yr, A.P. 9-1-46	"	"	100 1	2	3	2	0			0	80
M.P.	♂, 7 yr acute polio-myelitis 2-4-47, expired 2-5-47	2-4	" 96-7-8	0	0	0	0	0	0		0	0
		"	Normal	0	0	0	0	0	0		0	0

* A.P. = Anterior poliomyelitis.

† The virus preparation used, with the exceptions stated, was 91-2-4; the normal cotton rat antigen was the same as that described in the text.

tion had been demonstrated between virus and its antibody. However, unequivocal proof was provided when sera from a Lansing convalescent rhesus monkey and from 13 convalescent human cases† were examined for complement-fixing antibodies with concentrated Lansing virus and with the normal brain and spinal cord concentrate from cotton rats.

Blood was obtained from the monkey 3 weeks after it developed a partial paralysis following intracerebral inoculation with Lansing virus. The serum fixed complement when concentrated virus was used as antigen, giving a titer of 320. No fixation occurred when the concentrate from normal rat tissue was used as antigen, and sera from 6 normal monkeys were negative against both the virus and the antigen from normal rat tissues.

The results with 13 recent human convalescent sera and with that from a fatal case are recorded in Table IV. In those cases where positive complement-fixation was found, the tests were repeated several times with essentially identical results. Six of the 14 sera were positive, giving titers that ranged up to 320, seven were negative as was also that from the fatal case obtained the same day final diagnosis was made. In the positive tests there was no question about complement-fixation with a normal brain protein impurity in the concentrated virus since negative results were found when the preparation from normal rat tissues was used as antigen.

Second samples of blood from three subjects giving high titers were reexamined about 5 weeks after the first samples had been tested. As shown in Table IV, these again gave pos-

† The samples of monkey blood were kindly provided through the cooperation of Professor E. W. Schultz and Dr. S. C. White and the human convalescent sera by Drs. H. K. Faber, L. A. Luz, and R. D. Cutter.

TABLE V.
Comparison of Neutralization and Complement-fixation Tests with Human Sera.

	Serum	Dilution used	I.D. 50*	Neutralization titer†	Complement-fixation titer
M.K.	Convalescent	1:1000	$10^{-7.9}$	$10^{-4.9}$	320
R.He.	"	1:100	$10^{-9.0}$	$10^{-7.0}$	+
D.W.	"	"	$10^{-8.8}$	$10^{-6.8}$	0
M.P.	Fatal case	"	$10^{-10.3}$	$10^{-8.3}$	0

* 1 I.D. 50 = g of virus nitrogen required in a dose of 0.05 ml to infect 50% of animals when the dilution of serum given was used.

† Neutralization titer is defined as grams of virus nitrogen neutralized to the 50% end point by 1 ml of undiluted serum.

itive tests with titers that were comparable to those obtained the first time.

A comparison of the histories of the patients failed to show any correlation of positive or negative reactions with either age of the patient or length of time between onset of the disease and the testing of blood samples. The demonstration that approximately 45% of the group of patients possessed humoral antibodies that react with Lansing virus emphasizes the importance of this or a closely related strain as one responsible for poliomyelitis in humans. In the case of the negative reactions it is possible either that the individuals in question failed to produce humoral antibodies in sufficient concentration to be detected or that they may have been infected by a poliomyelitis virus immunologically unrelated to the Lansing strain.[9]

As shown in Table IV the samples of convalescent human sera showed a wide range in their complement-fixing titers. It was of interest to determine if complement-fixation titer could be correlated with ability to neutralize active virus. Neutralization tests were accordingly made on four human sera, namely the one giving the strongest reaction (M.K.), one showing weak complement-fixation (R. He.), and 2 giving no reaction (D. W. and M. P.). The tests were set up using a 1:100 or a 1:1000 dilution of serum in saline with serial dilutions of virus in steps of ten from 10^{-11} g nitrogen per ml to 10^{-5} g nitrogen per ml. The mixtures were incubated at 37° for one-half hour, and 0.05 ml of each dilution was injected intracerebrally into each of 5 young cotton rats. The infectivity of the mixtures was determined by the incidence of poliomyelitis and the amount of active virus necessary to infect 50% of the animals calculated according to the method of Reed and Muench.[10] The results which are recorded in Table V showed that over a hundred times as much virus was neutralized by serum M. K. (complement-fixation titer 320) as by either the weakly positive or the negative convalescent sera. Of interest was the fact that the sample from the fatal case, M. P., neutralized less than one-tenth as much virus as the negative convalescent serum. These results are, therefore, in agreement, with the view that the same antibody was involved in complement-fixation and in virus neutralization.

Conclusions. The experiments presented above show that complement-fixing antibodies can be demonstrated in the blood of rats immunized with formalized Lansing virus, in convalescent monkey serum, and in some convalescent human sera when concentrated Lansing virus from cotton rats is used as antigen. The results with rat sera are complicated by the presence of antibodies against components of normal brain and spinal cord in both immune and normal animals, but the evidence indicates that the primary reaction with immune sera is between antibody and virus. Immunization as a result of injection of formalized virus is, therefore, associated with the production of serologically detectable antibodies to active virus. The experiments with the convalescent monkey and human

[9] Sabin, A. B., *J. Am. Med. Assn.*, 1947, **134**, 749.

[10] Reed, L. J., and Muench, H., *Am. J. Hygiene*, 1938, **27**, 493.

sera provide definite proof that positive complement fixation can be demonstrated when concentrated Lansing virus prepared as described[2] is used as the antigen. These latter results further support the importance of the Lansing virus or an antigenically related strain as one responsible for the human disease.

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Influence of Pyribenzamine and Antistine upon the Action of Hyaluronidase.

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It is the general belief that the antianaphylactic and anti-allergic properties of Pyribenzamine and similar substances are due primarily to their ability to nullify the pathologic effects of histamine. This belief is based upon the following premises: First, that the symptoms of anaphylaxis and certain allergic diseases are produced by histamine, which is released during the antigen-antibody reaction; and secondly, that the most outstanding property of Pyribenzamine and related substances is their antihistaminic power (Mayer, Huttrer and Scholz,[1] Mayer[2]).

Many clinical observations on the activity of Pyribenzamine and similar substances in various forms of allergic manifestations appear to sustain this explanation. The effectiveness of antihistaminic therapy depends in a large measure upon the type of allergy against which it is used, and antihistaminics are most effective in conditions in which histamine release is suggested by experimental findings, namely in anaphylaxis, serum disease, urticaria or hay fever. On the other hand, if the activity of these substances is due solely to the inhibition of histamine, then they should be ineffective in allergic dermatitis or eczema, since none of the clinical symptoms of contact dermatitis or eczema appear to have any resemblance to the known histamine symptoms. To date, all attempts to produce these forms of skin reactions by the administration of histamine have been unsuccessful.

But in spite of these facts, there are numerous clinical reports citing the effectiveness of the various antihistaminics in contact dermatitis and atopic eczema (Feinberg,[3] Chobot[4]), and one of us has shown that Pyribenzamine exerts a considerable prophylactic activity in experimental contact dermatitis of guinea pigs (Mayer[5,6]). Since the effectiveness in these epidermal sensitizations is difficult to explain on the basis of inhibition of histamine, it appears more likely that antihistaminic substances act in these cases by virtue of a pharmacodynamic property different from their action upon proven histamine effects. All antihistaminic substances developed thus far possess, in addition to their antihistaminic power, various degrees of antispasmodic and local anesthetic activities. It is possible that the curative effect in dermatitis and eczema may be explained as being due to the local anesthetic property, although several observations contradict this view.

Recent investigations on the mechanism of epidermal inflammations due to the action of chemical or physical influences have pointed to the importance of certain amines, other than histamine, and of substances such as leukotaxine, pyrexin and necrosin (Menkin[7]). Although the significance of these substances

1 Mayer, R. L., Huttrer, G. P., and Scholz, C. R., *Science*, 1945, **102**, 93.

2 Mayer, R. L., *J. Allergy*, 1946, **17**, 153.

3 Feinberg, S. M., *J. A. M. A.*, 1946, **132**, 702.

4 Chobot, R., *J. Allergy*, 1947, **18**, 76.

5 Mayer, R. L., *J. Invest. Dermat.*, 1947, **8**, 67.

6 Mayer, R. L., *Ann. Allergy*, 1947, **5**, 113.

7 Menkin, V., *Arch. Path.*, 1946, **41**, 376.