A Critical Evaluation of the Indirect Basophil Degranulation Test.* (29650)

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Histamine is released from the tissue mast cell during an allergic reaction and its release is thought to be responsible for the positive skin test. Histamine is also found in the granules of the basophil leucocyte. As the basophil releases histamine during an allergic reaction, Shelley suggested that this phenomenon might be used to detect allergy(1). He and his colleagues first developed a "direct" technique to test for antibody in serum (1,2,3). A known antigen was added to the test serum. The leucocytes were concentrated by filtration and stained; the degree of degranulation of the basophils was then evaluated.

Because this procedure was tedious and time consuming and required the patient's own basophils, Shelley devised a new method to test serum for the presence of antibody (4). Rabbit blood was used as the source of basophils for an *in vitro* test. He claimed this "indirect" method was simple enough to lend itself to mass testing in a central clinical laboratory(4,5).

We planned to adapt this "indirect" basophil technique to the investigation of possible circulating antibodies in diseases such as rheumatic fever, glomerulonephritis and the collagen disorders. Before doing so we studied the specificity and reproducibility of the test; these studies form the basis of this report.

Material and methods. Serum was obtained from: (1) Three subjects who had had typical allergic reactions to penicillin respectively 14 days, 19 days and 2 years previously. (2) Three patients known to be allergic to ragweed and who had positive skin tests with ragweed antigen; these specimens were taken after the end of the ragweed season. (3) Two subjects with no history of allergy to ragweed or penicillin.

The sera were stored at —20°C for not more than 10 days before testing. The antigens used were powdered penicillin G‡ diluted 1:100 with 0.9% NaCl, and stored at 4°C; and commercial 1:100 ragweed antigen stored at 4°C.

Collection and handling of the rabbit blood and preparation of the basophil slide were done in the manner described by Shelley(4).

After we had familiarized ourselves with the technique, preliminary studies were done to learn to detect reactive preparations. We attempted to use Shelley's criteria(4) for a positive reaction: "The majority of basophils show a loss of sphericity, cell distortion, swelling, granulolysis, granular swelling, less staining, rapid oscillation or dancing of granules, loss of granules, appearance of clear cell nucleus, cell wall bleb formation, and streaming of granules from the cells." An attempt at quantitation of these observations revealed that all these findings could not be evaluated quantitatively and that they did not appear reliably to distinguish a reactive from a non-reactive preparation. In these preliminary studies on preparations of known composition we concluded that degranulation was the one index which could be examined quantitatively. When it occurred in more than 30% of the basophils examined the preparations were considered to be reactive, and were therefore called positive.

We also noted that if the composition of

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TABLE I. Reproducibility of Indirect Basophil Test.

Serum No.	Basophils + serum + antigen	Basophils + serum + saline	Basophils + saline + antigen
	Ragweed antigen Serum from ragweed sensitive subjects		
1	8/10*	3/10	1/10
2	6/10	7/10	0/10
Total	14/20	10/20	1/20
	Penicillin antigen Serum from penicillin sensitive subjects		
1	6/10	4/11	0/9
2	5/10	4/11	1/9
Total	11/20	8/22	1/18
Summary	25/40	18/42	2/38

* No. of preparations positive/No. of preparations studied.

the preparation were known, much prejudice was introduced in evaluating its reactivity. This potential source of bias was eliminated in all further studies.

The reproducibility of the test was investigated as follows: Two sets of experiments were done, the first with 2 sera from ragweed sensitive subjects using ragweed as antigen. With each serum, 30 slides were studied giving 10 replicate determinations. The slides were prepared by one of us in 3 series consisting of (1) rabbit basophils, ragweed sensitive serum and ragweed antigen; (2) rabbit basophils, ragweed sensitive serum and saline (serum control); and (3) rabbit basophils, ragweed antigen and saline (antigen control). Each slide was coded. The coded slides were read by a second observer. The code was broken only when all 30 slides from the 10 replicates in the experiment had been studied. The second experiment was done with penicillin as antigen and 2 sera from penicillin sensitive subjects.

Another study was done to determine whether normal serum would produce basophil degranulation. To preserve the "blind" nature of this study, normal serum and ragweed sensitive serum were studied in the same experiment. Eight replicates of each of 4 series of slides were prepared and coded by one observer: (1) Rabbit basophils, normal serum and saline; (2) Rabbit basophils, normal serum and antigen; (3) Rabbit basophils, ragweed sensitive serum and saline; (4) Rabbit basophils, ragweed sensitive serum and antigen. Again, the code was broken only when all 32 slides from the 8 replicates had been examined. A similar experiment was done using penicillin as antigen, a second normal serum, and serum from a penicillin sensitive subject.

Results. The data from the reproducibility study are summarized in Table I. The antigen control was read as negative in 36 of the 38 slides; the test system was read as positive in 25 of 40 slides, but the serum control was also positive in 18 of 42. The difference is not significant ($\chi^2 = 2.43$ $p > 0.10$).

The observations in the second experiment on normal serum are summarized in Table II. Normal serum and serum from penicillin and ragweed sensitive subjects could not be distinguished. The serum control was positive as frequently as the presumed reactive preparations.

Discussion. Shelley has claimed that the "indirect" basophil degranulation technique detects antibody in serum of penicillin reactors and that it may detect antibody to other foreign antigens(5). The results of our experiments suggest that Shelley's results could have occurred by chance.

Friedlaender and Friedlaender(6), using ragweed antigen to test blood from 32 known ragweed sensitive subjects, found positive basophil degranulation in only 8 and questionable results in another 13. They found difficulty in interpreting the results of the test because of a marked degree of degranulation in the serum controls, but not in the antigen controls. In this respect the results are similar to those shown in Table I. Katz,

TABLE II. Specificity of Indirect Basophil Test.

Source of serum	Basophils + serum + antigen	Basophils + serum + saline
	Exp 1—Ragweed antigen	
Healthy subject	5/8*	6/8
Ragweed sensitive subject	3/8	3/8
	Exp 2—Penicillin antigen	
Healthy subject	3/8	4/8
Penicillin sensitive subject	3/8	5/8

* No. of preparations positive/No. of preparations studied.

Gill, Baxter and Moschella(7) used the indirect basophil degranulation technique to study serum from 100 patients with a history of sensitivity to penicillin and from 56 control subjects with no such history. They, too, found frequent degranulation in the serum controls.

In examining Shelley's published observations it is clear that his experiments were done with adequate biological controls. It is not clear whether a serum control was run with every individual test. From our data and those of Friedlaender and Friedlaender and Katz *et al* it is evident that a serum control is necessary for each individual serum tested. The difficulties of reading the slides are such that prejudice is readily introduced. Thus positive results can only be accepted if the slides have been read double blind.

Summary and conclusions. The "indirect" basophil degranulation technique was evaluated by replicate "blind" analyses of 8 sera. The results indicate that degranulation of basophils was a random phenomenon which was not influenced significantly by the presence or the absence of the antigen in the system or by the presumed presence or absence of antibody in the serum.

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Nucleoside Phosphates in Liver of Mice During Experimental MHV-3 Virus Hepatitis.* (29651)

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Viral multiplication appears to be associated with the utilization of energy derived from oxidative phosphorylation. Anaerobiosis, reduction of oxygen consumption and dissociation of oxidative phosphorylation (obtained by different compounds devoid of virucidal activity), depresses the multiplication of certain viruses under different experimental conditions(1-5). However, in some strains of cells grown *in vitro* it has been possible to obtain active viral multiplication under anaerobic conditions(6). This is probably due to the high glycolytic activity typical of such cells grown *in vitro*. We have previously reported various metabolic modifications induced by viral multiplication in animals(7-10). This paper describes an investigation on nucleoside phosphates in the liver during experimental MHV-3 virus hepatitis in mice.

Methods. White mice, strain N.M.R.I., RECORDATI breed, weighing 12-16 g were fed with balanced synthetic diet "Altromin" of Altrogge Specialfutterwerk, Lage/Lippe, Germany. The MHV-3 virus (originally derived from the strain of Dr. Gledhill) was obtained from the ATCC. The following reagents were used: ATP-Test combination—"Enzymatische bestimmung von Adenosin-5'-triphosphorsäure"; ADP/AMP-Test combination—"Enzymatische bestimmung von Adenosin-5'-diphosphorsäure und Adenosin-5'-monophosphorsäure" of Biochemica Boehrin-

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