

BASOPHIL DEGRANULATION IN DRUG ALLERGY

By

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The human body is subjected to innumerable new drugs. It is no small wonder that quite a percentage of cases present with clinical signs and symptoms of drug eruption. Basophil degranulation test is one such method which is useful for determining drug allergy.

Shelley and Junlin (1961),^{1,6} Shelley (1962)^{1,8} and Shelley and Juhlin (1962) claimed that basophils of allergic patients degranulated when exposed to specific antigen *in vitro*. They described these test as Basophil Degranulation Tests. It was termed Direct Basophil Degranulation (DBDT) if the patient's own basophils were used; and called it indirect test if the patient's serum was mixed with normal basophils of rabbit and then exposed to specific antigen.

The present study reports our experience with Indirect Basophil Degranulation Test (IBDT) in drug allergy.

MATERIAL AND METHODS

The material in this study consisted of 29 cases of allergic reaction due to various drugs. It did not include such cases where reaction occurred more than 2 years ago. Twenty-five cases, who were receiving drugs but without any clinical reaction, constituted the control group.

In positive cases effect of 1% solution of corticosteroids on the degranulation response was studied besides the study of effect on degranulation after 15 days of treatment with corticosteroids in these cases. In addition to the actual test, the serum control with the buffy coat and drug control with buffy coat were done. Patient's with serum showing basophil degranulation (without drug) was discarded.

Technique: Shelley's technique for Indirect Basophil Degranulation Test was used.

OBSERVATIONS AND ANALYSIS

Observations are tabulated below :

TABLE No. I
Showing AGE Incidence

Age group	No. of caes		Percentage	
	Drug Allergy	Normal	Drug Allergy	Normal
0.10 years	2	2	6.90	8.00
11.20 years	1	2	3.45	8.00
21.30 years	9	8	31.04	32.00
31.40 years	10	5	34.48	20.00
41.50 years	3	5	10.34	20.00
51.60 years	3	2	10.34	8.00
61.70 years	1	1	3.45	4.00
Total	29	25	100.00	100.00

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TABLE No. II

Showing - Sex Incidence

Sex	No. of cases		Percentage	
	Drug Allergy Control	Control	Drug Allergy Control	Control
Males	18	18	62.07	72.00
Females	11	7	37.93	28.00
Total	29	25	100.00	100.00

TABLE No. III

Showing Number of cases with Types of Reactions

Type of reaction	No. of cases	Percentage
Immediate	7	24.14
Delayed	20	68.96
Immediate & Delayed	2	6.90
Total	29	100.00

TABLE No. IV

Showing the period elapsed after onset of reaction and the test.

Period elapsed after onset of reaction and the test.	No. of cases	Percentage
Within 2 hours	1	3.45
1-15 days	15	51.72
15-30 days	5	17.24
1-6 months	6	20.69
6 months-1½ years	2	6.90
Total	29	100.00

TABLE No. V-A

Showing incidence of positive indirect basophil degranulation test in various drugs.

S. No.	Name of the drug	Total No. tested.	Total No. positive
1.	Acetyl salicylic acid (Aspirin)	11	2
2.	Sulpha drugs	11	6
3.	Penicillin	9	5
4.	Streptomycin sulphate	6	-
5.	Isoniazid	5	-
6.	Thiocetazone	4	3
7.	Chloramphenicol	3	3
8.	Phenyl-butazone	2	2
9.	Procaine Hydrochloride	3	-
10.	Analgin	2	1
11.	Oxytetracycline hydrochloride	1	-
12.	Paracetamol	1	1
13.	Tolbutamide	1	1
14.	Chlorpropamide	1	-
15.	Ethinyl Oestradiol	1	-
16.	Methyl testosterone	1	-
17.	Amidopyrine	2	-
18.	Chloroquine sulphate	1	-
19.	Phenobarbitone sodium	1	-
20.	Caffeine	1	-
21.	Phenacetin	1	-
22.	Phenazone	1	1
Total.		69	25

TABLE No. V-B

Showing incidence of drugs used.

Name of the drug	Total No. tested	Results
Sulphadiazine	4	Negative
Penicillin G. sodium	4	Negative
Streptomycin sulphate	3	Negative
Tetracycline Hydrochloride	3	Negative
Acetyl salicylic acid	2	Negative
Chloramphenicol	2	Negative
Thiocetazone	2	Negative
Isoniazid	2	Negative
Analgin	1	Negative
Chlorpropamide	1	Negative
Paracetamol	1	Negative
Phenyl butazone	1	Negative
Ethinyl Oestradiol	1	Negative
Methyl testosterone	1	Negative
Phenobarbitone sodium	1	Negative
Total	29	

TABLE No VI

Showing Distribution of positive cases according to number of drugs

Drugs	Total cases	Positive cases
Single drug	5	4
Two drugs	16	11 (a)9 positive to one drug (b)2 positive to two drugs
Three drugs	4	4 (a)2 positive to two drugs (b)2 positive to two drugs
Multiple drug cases	4	2 (a)1 positive to two drugs (b)1 positive to one drug
Total	29	21

TABLE VII
*Showing the result of Indirect basophil degranulation test
 in single drug cases.*

S. No. Drug	Result of Indirect basophil degranulation test (IBDE)
1. Penicillin G. Sodium	Positive 48% degranulation.
2. Paracetamol	Positive 40% degranulation.
3. Streptomycin Sulphate	Negative 20% degranulation.
4. Penicillin G Sodium	Positive 80% degranulation.
5. Chloramphenicol	Positive 68% degranulation

TABLE No. VIII
*Showing the result of Indirect basophil degranulation test
 in two drugs cases.*

S. No. Drugs	Result of Indirect basophil degranulation test.
1. a) Acetyl salicylic acid	Negative 16% degranulation
b) Sulphadiazine	Positive 36% degranulation.
2. a) Chloramphenicol	Positive 52% degranulation
b) Oxytetracycline Hydrochloride	Negative 16% degranulation
3. a) Sulphamerazine	Negative 8% degranulation
b) Chloramphenicol	Negative 20% degranulation (Positive after 15 days 52% degranulation)
4. a) Sulphadiazine	Positive 48% degranulation
b) Acetyl salicylic acid	Negative 20% degranulation
5. a) Penicillin G. Sodium	Positive 40% degranulation
b) Sulphadiazine	Negative 8% degranulation
6. a) Potassium Phenoxymethyl penicillin.	Negative 20% degranulation
b) Sulphadiazine	Negative 8% degranulation
7. a) Sulphadiazine	Negative 12% degranulation
b) Acetyl salicylic acid	Negative 8% degranulation
8. a) Streptomycin sulphate	Negative 16% degranulation
b) Isoniazid	Negative 8% degranulation
9. a) Sulphadiazine	Positive 32% degranulation
b) Penicillin G. Sodium	Positive 36% degranulation
10. a) Chlorpropamide	Positive 36% degranulation
b) Tolbutamide	Positive 40% degranulation
11. a) Thiacetazone	Negative 20% degranulation
b) Isoniazid	Negative 20% degranulation
12. a) Phenylbutazone	Positive 32% degranulation
b) Aspirin	Negative 20% degranulation
13. a) Sulphadiazine	Negative 12% degranulation
b) Aspirin	Negative 12% degranulation
14. a) Thiacetazone	Positive 36% degranulation
b) Isoniazid	Negative 12% degranulation
15. a) Sulphadiazine	Positive 72% degranulation
b) Acetyl salicylic acid	Negative 20% degranulation
16. a) Amidopyrine	Negative 20% degranulation
b) Phenylbutazone	Positive 52% degranulation

TABLE No. IX
Showing the result of Indirect basophil degranulation test in three drugs cases.

S. No. Drugs	Result of Indirect basophil degranulation test		
1. a) Streptomycin sulphate	Negative	8%	degranulation.
b) Acetyl salicylic acid	Negative	12%	degranulation.
c) Sulphadiazine	Positive	48%	degranulation.
2. a) Analgin	Positive	32%	degranulation.
b) Procaine Hydrochloride	Negative	4%	degranulation.
c) Penicillin G. Sodium	Negative	8%	degranulation.
3. a) Thiacetazone	Positive	44%	degranulation.
b) Acetyl salicylic acid	Positive	48%	degranulation.
c) Isoniazid	Negative	16%	degranulation.
4. a) Penicillin G Sodium	Positive	60%	degranulation.
b) Analgin	Negative	16%	degranulation.
c) Acetyl salicylic acid	Positive	36%	degranulation.

TABLE No. X
Showing the result of Indirect basophil degranulation test in more than three drugs cases.

S. No. Drugs	Result of Indirect basophil degranulation test.		
1. a) Thiacetaxone	Positive	40%	degranulation.
b) Acetyl salicylic acid	Negative	4%	degranulation.
c) Chloroquine phosphate	Negative	4%	degranulation.
d) Streptomycin sulphate	Negative	4%	degranulation.
e) Isoniazid	Negative	8%	degranulation.
2. a) Ethinyl Oestradiol	Negative	12%	degranulation.
b) Methyl testosterone	Negative	4%	degranulation.
c) Phenobarbitone sodium	Negative	4%	degranulation.
d) Penicillin G. Sodium	Negative	8%	degranulation.
e) Procaine Hydrochloride	Negative	0%	degranulation.
d) Streptomycin sulphate	Negative	8%	degranulation.
3. a) Phenazone	Positive	40%	degranulation.
b) Caffeine	Negative	8%	degranulation.
c) Phenacetin	Negative	4%	degranulation.
d) Amidopyrine	Negative	4%	degranulation.
e) Sulphadiazine	Positive	36%	degranulation.
4. a) Streptomycin sulphate	Negative	8%	degranulation.
b) Procaine Hydrochloride	Negative	4%	degranulation.
c) Penicillin G. Sodium	Negative	12%	degranulation.
d) Acetyl Salicylic acid	Negative	12%	degranulation.

TABLE No. XI
Showing the comparison of the Indirect basophil degranulation test with the skin tests.

Type of test	Positive	Negative	Total
Indirect basophil degranulation test.	21	8	29
Skin tests.	15	14	29

Twenty nine cases of drug allergy have been included in this study.

The age group of these patients varied from 3 years to 70 years. About 2/3rd of cases came during the third and fourth decades. The detail of ages in the various age groups is given in Table No. I.

Sex incidence is given in Table No. II.

Time period elapsed after the onset of clinical reaction and the indirect basophil degranulation test varied from less than 2 hours to 1½ years. It is shown in Table No. IV.

Twenty two drugs were tested with indirect basophil degranulation test to find out whether they were responsible for producing the clinical reaction. The various drugs which were implicated and tested in these cases are given in Table No. V-A.

Positive cases according to the number of drugs is tabulated in Table No. VI. Out of 5 single drug cases, 4 were positive; 16, 2 drug cases showed 11 positive; 4, three drugs cases showed 4 positive; 4, more than three drugs cases showed 2 positive.

TABLE No. XII
Showing Indirect Basophil Degranulation Test Analysis of Control cases (Group II)

S. No. Drug	Result of indirect basophil degranulation test.
(Single drug cases)	
1. Sulphadiazine	Negative 4% degranulation
2. Sulphadiazine	Negative 8% degraulation
3. Sulphadiazine	Negative 4% degranulation
4. Sulphadiazine	Negative 4% degranulation
5. Penicillin G. Sodium	Negative 4% degranulation
6. Penicillin G. Sodium	Negative 8% degranulation
7. Penicillin G. Sodium	Negative 12% degranulation
8. Penicillin G. Sodium	Negative 8% degranulation
9. Streptomycin	Negative 4% degranulation
10. Streptomycin	Negative 12% degranulation
11. Streptomycin	Negative 4% degranulation
12. Tetra cycline hydrochloride	Negative 12% degranulation
13. Tetra cycline hydrochloride	Negative 16% degranulation
14. Tetra cycline hydrochloride	Negative 8% degranulation
15. Acetyl salicylic acid	Negative 12% degranulation
16. Acetyl salicylic acid	Negative 12% degranulation
17. Chloramphenicol	Negative 12% degranulation
18. Chloramphenicol	Negative 8% degranulation
19. Analgin	Negative 4% degranulation
20. Chlorpropamide	Negative 16% degranulation
21. Paracetamol	Negative 4% degranulation
22. Phenyl butazone	Negative 4% degranulation
(two drug cases)	
23. Thiacetazone	Negative 8% degranulation
Isoniazid	Negative 8% degranulation
24. Thiacetazone	Negative 4% degranulation
Isoniazid	Negative 8% degranulation
25. Ethinyl Oestradiol	Negative 0% degranulation
Methyl testosterone	Negative 4% degranulation
Pehno-barbitone sodium	Negative 8% degranulation

SERUM CONTROL

It was done in all the 29 cases and none of them showed a positive result. Twelve cases had 4% degranulation each and 17 had no degranulation.

DRUG CONTROL

Drug control was also done for all the drugs and not a single drug showed a positive test. All these drugs showed 0-4% degranulation except two instances where the degranulation was 8%. The average level of degranulation was 3.19%.

CONTROL STUDY OF 25 CASES

Indirect basophil degranulation test was done in 25 cases, who were receiving almost similar drugs but had no allergic reaction. The aim of this control study was firstly to compare the results of indirect basophil degranulation test with those of drug allergic individuals, secondly to assess the value of this test in forestalling (anticipating) any allergic reaction in an individual without clinical manifestation of drug allergy. Results of this control study are tabulated in Table No. I, II, V-B. The indirect basophil degranulation test was negative in all the 25 cases of control study.

INDIRECT BASOPHIL DEGRANULATION WITH ADDITION OF PREDNISOLONE SOLUTION ON THE SLIDE.

It was carried out in 21 positive cases only. A marked decrease in the percentage of degranulation was observed. The degranulation ranged from 4% to 24% with an average of 12%.

INDIRECT BASOPHIL DEGRANULATION TEST AFTER TWO WEEKS THERAPY WITH PREDNISOLONE

It was done in all the 29 cases and observed that there was only negligible decrease in the percentage of degranulation without appreciable affecting the results. In 43 negative drugs the degranulation ranged from 0-20% with an average of 9.25%; whereas in positive drugs, the degranulation ranged from 28% to 60% with an average of 40%.

DISCUSSION

Basophil degranulation test has received a good deal of attention in comparatively recent years. It has been used by various authors in the different aspects of drug allergy and has proved to be quite useful. Thus, an added importance has been attached to Indirect Basophil Degranulation Test. If this procedure is really useful as reported by various authors, it is imperative to evaluate this test and evolve the best possible technique for this. This problem was undertaken in this institution with this object in mind and our results amply project the conclusions drawn therefrom.

SERUM

During this study serum has always been used within two hours of the collection of blood. We fully subscribe to the view that use of fresh serum is definitely a better procedure than using the stored one. However, in unavoidable circumstances, serum stored at -20° C as advocated by Schawartz et al. (1965)^{1, 2} and Hellman et al (1966)⁵ could be used. In this series, there has never been an occasion of using stored serum. A fresh serum, collected in this way, never gave more than an occasional degranulated cell in serum controls of this series. It is further suggested that if, per chance, in a serum specimen there is more than an occasional degranulation, a fresh sample of serum should be taken. A positive indirect basophil degranulation test in serum control always vitiates the value of this test.

DRUG

Chemically pure samples of drugs should be used as they are free from excipients. The sterile saline solution is quite good for diluting the drugs, using their exact weight and volume as reported by Shelley (1962)^{1, 3}; O' Quinn (1965)⁶; Gordon (1966)⁴; Hellman et al (1966)⁵. There has been some controversy over the use of saline where the drug is insoluble in it. Some authors, Gokhale and Joglekar (April, 1964)³ have used other solvents like ether, acetone and alcohol; but their use, will induce the basophil degranulation due to their toxic effects on basophils. We, too, are not in favour of these solvents. Sterile, normal saline has consistently given good results in this series, as well as in those of other workers including Shelley. As regards the drug dilution, wherever explosive degranulation with 1/100 dilution was encountered, the test was repeated with 1/1000 dilution. In drug controls of this series, we never encountered more than one or two degranulated cells out of 25 cells counted for the purpose of this test.

STAIN

Slides were stained with 0.05% of supravital neutral red solution. Our experience with stored stock solution has not been good, and it changed its colour on keeping it for more than 24 hours. Hence, in this series, the stain solution was always prepared afresh.

RABBIT

Selection of the rabbit is also important. Collection of blood is described. We have used, small sized polythene tubes for the separation of buffy coat as has been done by other workers like Shelley (1962)^{1, 3}; Freidlander and Freidlander (1964)². During this study, a centrifuge with 6000 revolution per minute has been used. It is spun for 10 minutes and has effected quite a satisfactory separation of buffy coat whereas most of other authors have used micro-haematocrit centrifuge with 11500 revolutions per minute spun for 30 seconds. Our experience, with a centrifuge having 6000 revolutions per minute adds to the usefulness and practicability of this procedure because it will be possible to carry out this test in small hospital where a micro-centrifuge may not be available.

A control study of 25 cases, who were receiving almost similar drugs but without any allergic reaction, has also been carried out. The aim behind this control study has been two fold. Firstly, to compare it with those of allergic individuals; and secondly, to assess the value of this test, to forestall or anticipate allergic reaction in an individual. None of the control cases turned out to be positive. Therefore, it is concluded that this cannot predict the allergic reaction in and given cases. The test in our opinion can only tell about the present allergic status of the individual.

The criteria for assessment of positive results have also differed with various authors as given below :—

S. No.	Authors	Criteria of Assessment
1.	Shelley and Caro (1962) ¹⁵	20% cells degranulated proved that it is 4 times the cells degranulated in control.
2.	Shelley (1962 ¹³ and 1963 ¹⁴)	Counting of 20 basophils with 30% or more abnormal cells.
3.	Friedlaender and Friedlaender (1964) ²	50-100 cells counted. The test positive if more than 30% cells degranulated.
4.	Katz et al (1964) ⁶	20 cells counted. Abnormal basophils in actual test—abnormal basophils in serum control = TSC value. TSC value of 6 or more = + (positive). TSC value of 4 or 5 = \pm TSC value of 3 or less = - (negative).
5.	Kravis et al (1965) ⁷	20 cells counted. The test is positive if degranulation is at least 25% more than controls.
6.	Schwartz et al (1965) ¹¹	40 cells counted. The test is positive if degranulation is atleast double the serum and antigen control.
7.	Gordon (1966) ⁴	30 cells counted. Test is positive if 30% or more degranulated cells.
8.	Hallman et al. (1966) ⁵	40 cells counted. Test is positive if degranulation is atleast twice or more than the controls.
9.	Present series	25 cells counted. Test is positive if 30% or more cells degranulated.

It will be clear from the above table that the majority of the workers have quoted 30% degranulation as an effective index for considering a positive result. Whether it is the control slide or the actual test slide, we are in full agreement with the above observations. As regards the number of cells counted, there has been variation from 20-100 cells to reduce the percentage of error. Counting of 25 cells is considered by us to be a reasonable count and has proved to be quite satisfactory being less time consuming.

Indirect basophil degranulation test has been done in 29 cases. Most of the cases came either during the reaction or within the first few days of reaction and only a few after one month of the reaction. All the patients, included in this series, came within the first 2 years of the reaction. Some of these patients had taken one drug, others two and the remaining more than two.

Percentage of positive cases is 72-41%. It is clear from our observation that we obtained about 73% positive results from the indirect basophil degranulation test. Shelley (1962¹³) reported about 64%; Siddi *et al.* (1963¹⁷), reported 11 positive cases out of 13 with 84.6% positive results, Friedlaender and Friedlaender (1964²) gave 36%; Brannen and Forbes¹ 67%; Katz *et al.* (1964⁶) 65%; Palmeque *et al.* (1965⁹) reported 34% positive results, Hellmen *et al.* (1966⁵) gave about 60% positive results; Gordon (1966⁴) gave 76% positive results. It is obvious from the above that there is a wide variation in the percentage of positive results obtained by different authors. There could be so many reasons for this variation. Firstly, there is always variation regarding selection of cases. Secondly, frequent, positive serum and drug controls may vitiate the quantitative value of the test as has already been observed by Friedlaender and Friedlaender (1964²). Thirdly, it is possible that the patient might have become sensitive to some breakdown product of the drug i. e. drug metabolite or hapten-protein combination and not to the pure drug itself. Therefore, a negative indirect basophil degranulation test could be due to the patient being allergic to the drug metabolite rather than the drug itself. Fourthly, as pointed out by Samitz *et al.* (1965¹⁰) there is a refractory period immediately after a severe drug reaction and the test may be negative during this period. It is significant because if the patient is given drug during this period, there may be no reaction. In our series, there was a case with negative Indirect Basophil Degranulation test who came during anaphylactic reaction after taking sulpha and chloromycetin drug. The test was negative for both the drugs at that time. When the test was repeated after two weeks for follow up study, it became positive to chloramphenicol and negative to sulpha drug, although the patient was on prednisolone therapy during this period. Fifthly, cases with history of drug reactions of more than 2 years back, will show negative results as their antibodies get gradually depleted. If the patient is not in the refractory period, the negative test indicates only the absence of antibodies on the day of the test. It does not predict the situation likely to appear after a week or more. The patient could develop antibodies later and have an allergic reaction. In a carefully standardised and executed test, the chances of false positive or false negative should not be there.

EFFECT OF CORTICOSTEROIDS

We substantiate the view of Shelley (1963¹⁴) in finding out an immediate blocking effect of corticosteroids (prednisolone) on the antigen-antibody reaction. A positive test became negative on addition of prednisolone on the slide. In clinical practice, although the corticosteroids are quite effective in the amelioration of an allergic reaction, it has been observed that when the

serum sample of a patient on corticosteroids therapy for two weeks is used, there is only slight decrease in the percentage of degranulation as compared to what we observe in our in-vitro studies. It appears that the concentration of corticosteroids in the invitro test is much more than that in the serum of a patient on corticosteroid therapy and that is probably the cause of discrepancy in the two facets of this test. However, this problem requires further detailed study and elucidation.

In the end, we conclude that the indirect basophil degranulation test is technically sound, safe, simple, easily workable and relatively rapid procedure both sensitive and specific in vitro test.

It has been specially valuable in patients with allergic reaction after multiple drugs. The offending drug could be detected by Indirect Basophil Degranulation Test with some certainty, although a negative basophil degranulation test does not exclude a particular drug allergy.

Our experience with this test has been very encouraging and we strongly recommend its use in detecting drug allergy.

REFERENCES

1. Brannen, M. and Forbes, M. A. : Quoted by Shelley, W. B. : Further experience with the indirect basophil test. *Archives of Dermatology*, 91 :165-170, 1965.
2. Friedlaender, S. and Friedlaender, A. S. : Observations on basophil degranulation as an indicator of antigen-antibody reaction. *Journal of Allergy*, 35 :361, 1964.
3. Gokhle, B. B. and Joglekar, M. V. : Lepra reactions and basophil degranulation test. *The Indian Practitioner*, 17 : 377-378, 1964.
4. Gordon, V. H. : The indirect basophil test and drug hypersensitivity. *Annals of Allergy*, 24 : 171-178, 1966.
5. Hellman, D. M., Klopstock, A., Schwartz, A. and Vardinon, N. : The basophil degranulation test in patients hypersensitive to drugs. *Acta Allergologica*, 21 : 17-24, 1966.
6. Katz, H. I., Gill, K. A., Baxter, D. L. and Moschella, S. L. : Indirect basophil degranulation test in penicillin allergy. *Journal of American Medical Association*, 188 : 351-354, 1964.
7. Kravis, L. P., Lecks, H. I. and Whitney, T. : Basophil degranulation test in atopic allergic states. A pilot study of ragweed pollen sensitive patients. *Journal of Allergy*, 36 : 23-28, 1965.
8. O'Quinn, S. E. : The use of the indirect basophil degranulation test in the investigation of drug allergy. *Southern Medical Journal*, 58 : 1147-1151, 1965.
9. Palomeque, F. E., Fulton, J. and Derbes, V. J. : Penicillin Sensitivity. *Archives of Dermatology*, 92 : 271-275, 1965.
10. Samitz, M. H., Simons, H. M. and Shelley, W. B. : Anaphylactic reaction to commercial ACTH of porcine origin. Results of basophil degranulation test. *Annals of Allergy*, 23 : 51-53, 1965.
11. Schwartz, J., Klopstock, A., Duvdevani, P. Z. and Honsg, S. : Detection of hypersensitivity by indirect rat mast cells degranulation. *International Archives of Allergy and Applied Immunology*, 26 : 333-339, 1965.
12. Schwartz, J., Klopstock, A. and Vardinon, N. : The role of the complement in the basophil cell test. *International Archives of Allergy and Applied Immunology*, 26 : 142-152, 1965.
13. Shelley, W. B. : New Serological test for Allergy in man. *Nature*, 195 : 1181-1183, 1962.
14. Shelley, W. B. : Indirect basophil degranulation test for allergy to penicillin and other drugs. *Journal of American Medical Association*, 181 : 171-178, 1963.
15. Shelley, W. B. and Caro, W. A. : Basophil degranulation in anaphylaxis. *Journal of American Medical Association*, 182 : 172-178, 1962.
16. Shelley, W. B. and Juhlin, L. : A test for detecting anaphylactic sensitivity. The basophil reaction. *Nature*, 191 : 1056-1058, 1961.
17. Siddi, E.; Reinberg, A. and Gervais, P. (1963). : Quoted by O'Quinn, S. E. : The use of the indirect basophil degranulation test in the investigation of drug. 58 : 1147-1151, 1965.