METHYSERGIDE INDUCED DEGRANULATION OF THE BASOPHIL LEUKOCYTE IN MAN*

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The disappearance of the circulating basophil leukocyte in allergic reactions in man has been described in urticaria, anaphylaxis (1), food sensitivity and drug reactions such as penicillin and novobiocin (2, 3). Moreover, *in vitro* degranulation of the basophil has been well studied and documented in allergic states (4). The state of the basophil in non-allergic conditions is less well known, although recently the basophil has been profiled for systemic medical conditions, as well as for contact dermatitis (5, 6).

Although allergic drug reactions are associated with degranulation and disappearance of the basophil, less is known concerning the pharmacologic effect of drugs on this cell in man. Steroids in high dosage cause a delayed basopenia (7). Histamine liberators, *e.g.* 48/80, are also known to lower the basophil count (8). Estrogens and androgens were without effect, whereas progesterone may cause a decrease in circulating basophils (9). Other hormones were without pronounced effects (10). In studies on rabbits, drugs have produced variable responses (11).

The present study was undertaken as a systematic survey of the effect of commonly used drugs on the morphology and number of circulating basophils in man. It was hoped to find a drug capable of slowly and completely degranulating the circulating basophils and thereby depleting them of histamine. Such a compound would have considerable prophylactic value in the treatment of highly sensitized individuals in whom the explosive degranulation and release of histamine from the basophil and associated mast cell have the potential of shock and sudden death (12).

METHOD

Groups of five healthy Caucasian and Negro adult males received medication orally in one dose. Ten cc of heparinized blood for absolute basophil counts (ABC) by the chamber technic were drawn before medication, and 4 hours, 24 hours, and 48 hours after medication. The basophil count was performed immediately. Subjects were not used on successive weeks.

Equipment

(1) WBC pipettes
(2) Eosinophil counting chamber (Spiers-Levy)
(3) Diluting fluid
    25 cc stock dye solution
    7 cc hemolyzing solution
a) Stock dye solution
    50 mgm Toluidine Blue
    22 cc of absolute ethyl alcohol
    100 cc of 0.9% sodium chloride solution
b) Hemolyzing solution
    Saturated solution of saponin (J. T. Baker Chemical Co.) in 50 cc of ethyl alcohol and 50 cc of distilled water.

This is a modification of Kovac's technic (13). It was found that too little saponin would not hemolyze the red blood cells. Seven cc of hemolyzing solution added to 25 cc of stock solution gave excellent visualization of the basophil. The diluting fluid should be filtered daily and fresh hemolyzing solution and diluting fluid be used every 30 days.

Lichtgrün was also omitted from the solution, as eosinophil morphology was not recorded.

NOTE: Room temperature of 72° F. is necessary. At higher temperatures, saponin in the diluting fluid precipitates in the counting chamber within minutes preventing basophil determinations.

(4) Vacutainer®, B-D 3200 KA (Becton, Dickinson and Company)

Procedure

(1) Draw blood to 1.0 mark in WBC pipette. Add diluting fluid and continue drawing to 11.0 mark. (1:10 dilution)
(2) Shake 30 seconds on pipette shaker, or 2 minutes manually.
(3) Discard first 4 drops from pipette.
(4) Fill each of the 4 chambers on counting chamber.
(5) Place chamber in covered petri dish on moistened filter paper. Let sit for 10 minutes to allow staining and settling of the cells.

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TABLE I

Single Dose Drug Effect

(Data averaged for 5 subjects)

Drug	Dosage		Zero Hour	4 Hrs.	24 Hrs.	48 Hrs.
No medication		Degranulation	17%	27%	18%	13%
		Absolute Basophil Count (ABC)	48	52	41	54
Acetyl salicylic acid	30 gr	Degranulation	22%	30%	28%	28%
		ABC	37	42	45	41
Aqueous aspirin (Alka Seltzer®)	10 gr	Degranulation	2%	10%	7%	5%
		ABC	53	51	59	60
Aminopterin	0.5 mgm	Degranulation	31%	31%	30%	17%
		ABC	67	64	55	62
Calcium lactate (placebo)	10 gr	Degranulation	6%	8%	5%	3%
		ABC	33	30	25	26
Chlordiazepoxide hydrochloride (Librium®)	20 mgm	Degranulation	28%	37%	38%	35%
		ABC	32	34	35	35
Chloramphenicol (Chloromycetin®)	500 mgm	Degranulation	11%	20%	26%	23%
		ABC	61	60	60	59
Chloroquine (Aralen®)	500 mgm	Degranulation	15%	47%	56%	53%
		ABC	67	66	58	59
Dexchlorpheniramine maleate (Polaramine®)	6 mgm	Degranulation	13%	14%	19%	9%
		ABC	38	43	45	45
Dextroamphetamine (Dexedrine®)	15 mgm	Degranulation	29%	43%	44%	39%
		ABC	57	60	59	66
Ethinyl estradiol (Estinyl®)	0.5 mgm	Degranulation	19%	23%	30%	31%
		ABC	37	46	49	39
Griseofulvin (Grisactin®)	500 mgm	Degranulation	14%	22%	43%	48%
		ABC	65	45	47	42
Guanethidine (Ismelin®)	20 mgm	Degranulation	20%	20%	25%	29%
		ABC	51	62	59	53
Isoniazid	1 tablet	Degranulation	17%	17%	8%	17%
		ABC	60	53	54	50
Lactose (placebo)	10 gr	Degranulation	8%	25%	22%	14%
		ABC	48	43	47	58
Liothyronine (Cytomel®)	25 mcg	Degranulation	14%	9%	15%	19%
		ABC	34	40	35	31
Meprobamate (Miltown®)	800 mgm	Degranulation	14%	17%	25%	19%
		ABC	46	37	39	46

TABLE I—*Continued*

Drug	Dosage		Zero Hour	4 Hrs.	24 Hrs.	48 Hrs.
Methysergide maleate (Sansert®)	4 mgm	Degranulation	16%	32%	65%	69%
		ABC	31	25	32	35
Norethindrone acetate (Norlutate®)	10 mgm	Degranulation	16%	39%	50%	55%
		ABC	60	56	56	64
Novobiocin sodium (Albamycin®)	250 mgm	Degranulation	14%	7%	18%	30%
		ABC	38	36	32	29
Phenylbutazone (Butazolidin®)	100 mgm	Degranulation	24%	30%	30%	25%
		ABC	53	56	45	51
Polymixin B sulfate	50 mgm	Degranulation	9%	13%	10%	3%
		ABC	38	44	32	43
Reserpine (Serpasil®)	2 mgm	Degranulation	31%	28%	20%	19%
		ABC	32	28	27	29
Triamcinolone (Aristocort®)	4 mgm	Degranulation	12%	48%	83%	42%
		ABC	39	37	34	36
Trimethadione (Tridione®)	300 mgm	Degranulation	21%	36%	35%	39%
		ABC	49	51	51	50
Vitamin A (Aquasol A®)	50,000 units	Degranulation	29%	31%	36%	20%
		ABC	48	50	47	44

(6) Read under high power (430 ×). Count every square in each of the four chambers.
(7) Total the 4 counts, multiply this number by the correction factor 5 and then divide by the factor 4 (number of chambers). Report results per cu mm of blood.

The basophil count is read with the bright light of A-O Spencer microscope at 430 × (eyepieces 10 ×, objective 43 ×) using a didymium filter (Corning Glass, CS 160). Total time from bloodletting to completion of count is less than thirty minutes. Basophil morphology, as well as number, is recorded.

Normal basophils and degranulated basophils are classified. The percentage of basophil degranulation is determined by the per cent of the basophils which show degranulation.

Classification of Basophils

To facilitate a standard means of classification two categories are used: normal basophils and degranulated basophils. Normal cells have intense pink to purple stain, numerous small, reddish-purple granules located centrally and distinct cell outline.

Abnormal cells have light or faded pink stain, only two to five granules centrally or peripherally with central clearing. The granules not only decrease in number but become swollen as well. Vacuoles can be seen centrally and cellular outline becomes indistinct with cell shrinkage. Degranulation may be seen with granules extruding from the cell.

RESULTS AND DISCUSSION

Single Dose Drug Effect (Table I)

In repeated basophil counts of treated and untreated individuals, the absolute number of basophils remained relatively constant. No medication was found capable of simulating anaphylactic or acute urticarial reaction during which few circulating basophils remain.

It was possible, however, to induce striking cytologic changes in the basophil. These changes were noted in the cells as lessening in staining quality, reduced number of intracellular granules, centrifugal gathering of granules rather than central clumping, appearance of vacuoles within the cell, and even extrusion of granules when the basophil has ruptured. An arbitrary value of 0–30% basophil degranulation was considered normal. Above 30% was considered an abnormal response. The percentage of basophil

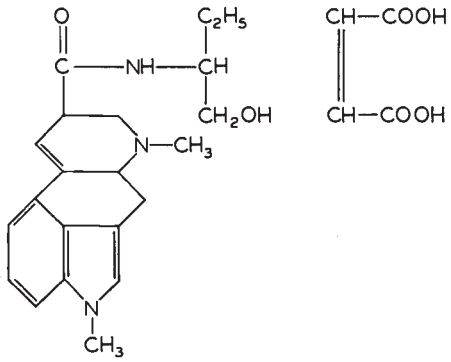

FIG. 1. Chemical structure of methysergide maleate (Sansert®, Deseril®).

degranulation following a single dose of medication can be seen in Table I. Groups treated with placebo medications (calcium lactate and lactose), as well as one group with no medication, showed a normal basophil picture over the entire 48 hour period. Most drugs tested compared closely with these findings. In 26 test situations only 5 were seen to induce noticeable morphologic change in the basophil. These were chloroquin, dextroamphetamine, methysergide maleate, norethindrone and triamcinolone.

Our findings of degranulation with triamcinolone agrees well with recent work (7). This may explain in part the beneficial results of steroids in reducing or preventing symptoms asssociated with drug allergy. One might also consider that the known anti-inflammatory effect of chloroquin is also related to this degranulation property. The effect of dextroamphetamine was unexpected and might well be studied further. The results with the progestational compound, norethindrone, are in agreement with the previous observation (9) that progesterone may cause some basopenia. Degranulation by methysergide was more marked than other drugs (Fig. 2, 3).

Long-Term Drug Effect (Table II)

Having shown some effect with a single dose, the question arose: "Would long-term drug therapy with maintained high blood levels be more damaging to the basophil?" To attempt to answer this question, 5 drugs were studied. Two of these were shown in Table I to have no effect—dexchlorpheniramine and chloramphenicol. The other 3—methysergide, norethin-

TABLE II

Long-term Drug Effect

(Data averaged for 5 subjects)

Drug	Dosage	Zero Hour	4 Hrs.	Days						
				1	2	3	4	5	6	13
Chloramphenicol (Chloromycetin®)	250 mgm four times daily									
	Degranulation	5%	2%	15%	20%	21%	4%	6%	6%	9%
	Absolute Basophil Count (ABC)	34	32	40	39	38	41	37	34	30
Dexchlorpheniramine maleate (Polaramine®)	8 mgm twice daily									
	Degranulation	9%	10%	24%	37%	36%	11%	18%	33%	18%
	ABC	41	50	47	49	47	47	41	41	44
Methysergide maleate (Sansert®)	2 mgm three times daily									
	Degranulation	3%	26%	46%	65%	93%	93%	87%	83%	83%
	ABC	48	50	49	53	52	56	51	56	58
Norethindrone acetate (Norlutate®)	5 mgm twice daily									
	Degranulation	3%	38%	33%	66%	58%	47%	36%	25%	32%
	ABC	33	41	33	39	42	37	33	36	32
Triamcinolone (Aristocort®)	4 mgm four times daily									
	Degranulation	4%	19%	52%	36%	34%	55%	42%	29%	26%
	ABC	28	26	28	27	26	28	32	27	23

drone and triamcinolone—were effective in producing some degree of degranulation after 4 hours and 24 hours (Table I), and might represent drugs capable of inducing 100% basophil degranulation and basopenia.

Again methysergide (Fig. 1) showed pronounced degranulation, more dramatic than any of the other medications tested. Within 24 hours 46% degranulation had occurred, later reaching a peak level of 83–93% degranulation. Chloramphenicol and dexchlorpheniramine had no significant degranulation effect. Norethindrone and triamcinolone showed a mild degranulating effect, but this was not sustained. There was no basopenic response concomitant with degranulation.

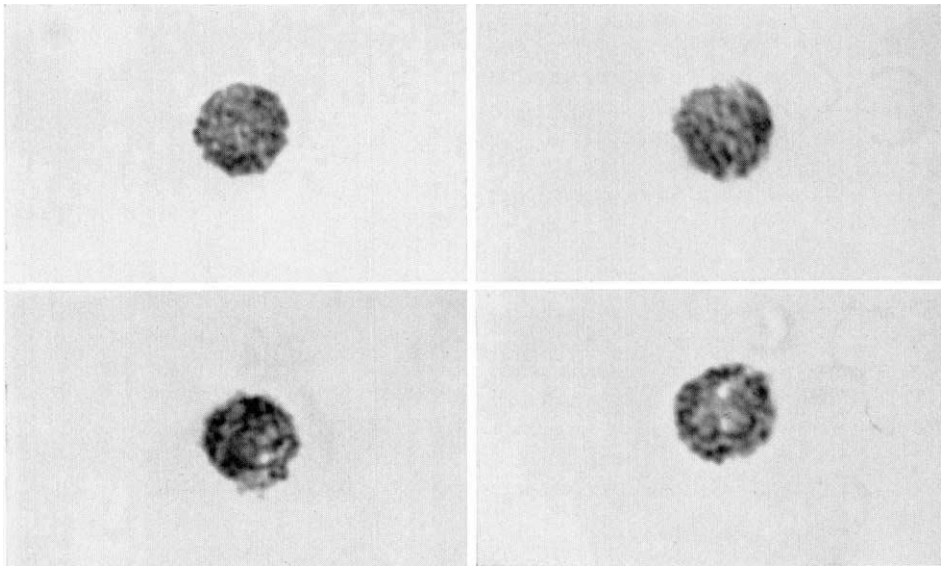

FIG. 2. Appearance of circulating basophil leukocytes of Subject 2 in counting chamber. Blood was drawn 4 hours after a single, oral dose of placebo. (Magnification 1750 ×)

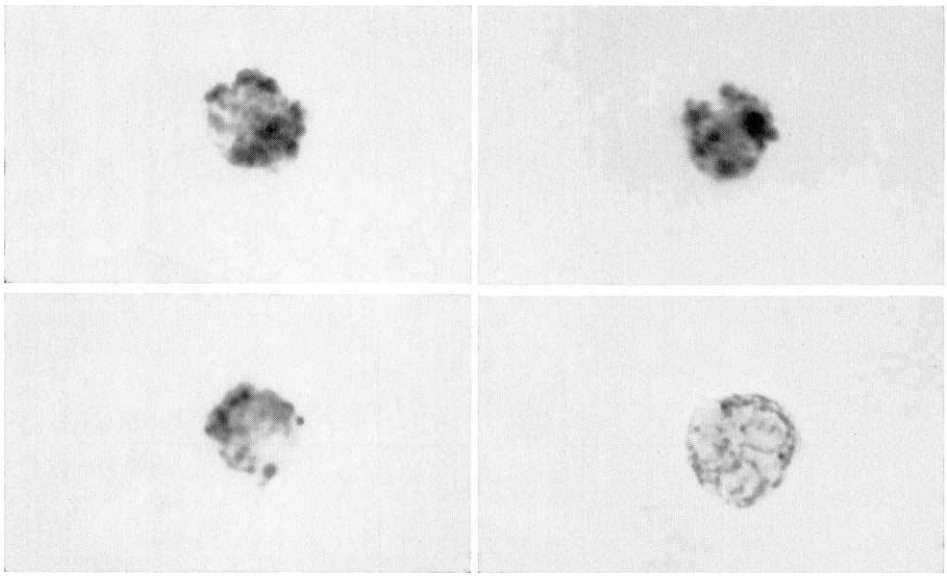

FIG. 3. Appearance of circulating basophil leukocytes of the same Subject 2, 4 hours after a single, oral dose of 4 mgm Methysergide. (Magnification 1750 ×)

Further Studies with Methysergide Maleate (Sansert) (Fig. 4, 5)

To further study methysergide, a new group of subjects was given the drug in increasing daily dosage. Side effects were lessened considerably by administration with food. Nevertheless, transient changes were noted by 4 of 5 subjects. On day 5, the circulating basophils became smaller. Figure 4 reflects per cent degranulation reaching a maximum at the peak dose of 12 mg daily and falling sharply as the dose was decreased on day 15. We were unable to cause 100% degranulation. Apparently mature basophils can be degranulated but immature basophils in their "green" state are less susceptible to degranulation. Slight change was noted in the absolute number of basophils (Fig. 5). In instances a substantial increase in the absolute count was seen. This can be explained by a rapid outpour of new immature basophils from the bone marrow.

Methysergide is well known to produce side effects (15). Employing a low dosage schedule of 2 mg twice a day with meals and increasing gradually, side effects can be lessened, but not completely eliminated. Certain subjects are more susceptible, and in these one sees nausea, paresthesias, leg cramps, flushing and vertigo. In our series, these were transient, lasting only several hours.

Methysergide disruption of basophil morphology has been more dramatic and more rapid than any drug in our series. Many cells were seen extruding granules in a spray like pattern. This relatively new compound is highly effective for the prophylactic treatment of vascular headache, such as migraine and cluster headache (histamine cephalgia) (16). It has not been effective in management of the acute attack. Chemically, it is 1-methyl-D-lysergic acid butanolamide, a derivative of lysergic acid and a potent serotonin antagonist (Fig. 1). It has been shown to block both serotonin dilatation and active components of reflex vascular dilatation (17). We have shown an effect on the basophil and we may assume the same effect on the mast cell, causing a gradual, slow degranulation. Certainly, in keeping with this view, Wolff and his group (16) have shown that methysergide (UML-491) can produce a marked reduction in the inflammatory response of human skin.

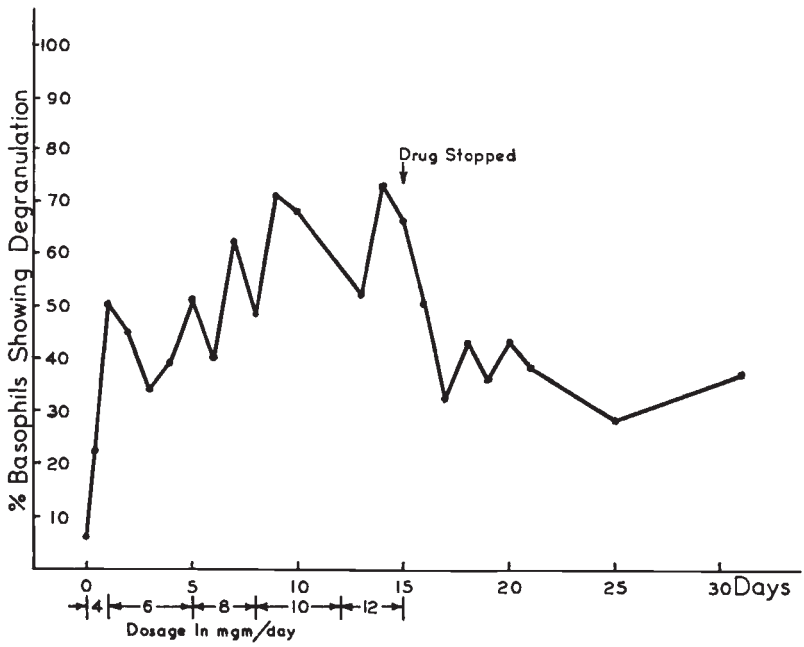

FIG. 4. Methysergide-induced degranulation: increase in percentage of basophils showing degranulation during administration of methysergide daily with meals. Dose increase caused gradual increase in degranulation. Note sudden decrease in degranulation response as medication is withdrawn. The graph gives data averaged for 5 subjects.

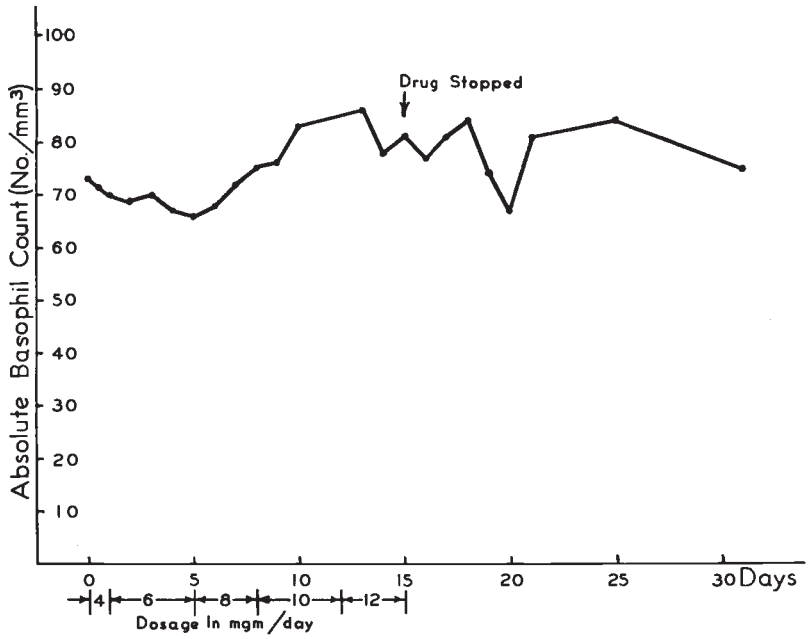

FIG. 5. Daily methysergide administration had no appreciable effect on the absolute basophil count. Data represents average for 5 subjects.

We suspect that at least part of the known benefits of methysergide in the prophylactic treatment of migraine and histaminic headaches results from its effect on the basophil. It would appear that the basophil-mast cell plays a significant role in the migraine syndrome. Triggered by various stimuli it may unload its histamine and heparin. In support of such a hypothesis, the blood coagulability has been shown to be sharply reduced during migraine attacks (18). The role of the basophil, as a source of bound histamine, is even more evident in histamine headache. In this instance numerous antigens may trigger basophil and mast cell degranulation with a subsequent histamine headache (19).

We feel that methysergide offers the migraine patient help by exhausting or depleting the basophil-mast cell stores of histamine. The continuous daily maintenance dosage is necessary to keep this cell system effete. As long as the basophil-mast cell is unable to respond to stress or allergens, the migraine attacks are lessened or eliminated.

By analogy, we feel that methysergide may offer the highly allergic individual relative protection from allergen triggered explosive release of basophil-mast cell stores of histamine. Some unpublished animal studies support this (20). For example, methysergide in a single dose of 50 mg/kg intraperitoneally protected 6 of 8 egg albumin sensitized guinea pigs from fatal anaphylactic shock.

The possibility that regular long-term daily dosage of methysergide will afford protection to patients highly sensitized to bee stings, penicillin, radio-opaque dyes, or food allergens deserves appropriate study.

SUMMARY

Twenty-five drugs were studied for effects on the morphology and number of circulating basophil leucocytes.

Five of the drugs, triamcinolone, chloroquin, methysergide, dextroamphetamine, and norethindrone, were found to cause significant degranulation of the basophils. No effect on the absolute basophil count was noted.

Methysergide proved to be the most effective of the five drugs. In dosage of from 2 to 12 mgm a day, it induced striking basophil changes.

It appears possible that the basophil-mast cell plays a significant role in the etiology of migraine and histamine headaches. Methysergide is viewed as preventing attacks by

depleting the basophils of their histamine-heparin granules.

It is suggested that methysergide be considered as a means of safe yet rapid "pharmacologic hyposensitization" of the highly allergic individual.

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