

Detection of IgE Antibodies Specific for 1-Phenyl-2,3-dimethyl-3-pyrazoline-5-one by RAST: A Serological Diagnostic Method for Sensitivity to Pyrazoline Drugs

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Pyrazoline drugs include phenazone (antipyrine), propyphenazone, aminophenazone (pyramidon) and metamizol (novalgin). The common chemical structure of these drugs is 1-phenyl-2,3-dimethyl-3-pyrazoline-5-one and their differences in chemical structure only involve the side chain (R) (Fig. 1). However, clinical use of aminophenazone was sharply curtailed after its potentially fatal bone-marrow toxicity, agranulocytosis, was recognized and extensively studied.¹⁻³ Aminophenazone was withdrawn in Sweden, UK, Germany, USA and China. Phenazone has also lost favor. Both drugs have disappeared from the therapeutic scene in USA, but phenazone is still employed in some countries, usually in analgesic mixtures. Metamizol and propyphenazone remain popular as analgesics.⁴

Besides bone-marrow toxicity, more attention has been paid to sensitive reactions caused by pyrazoline drugs including special symptoms varying from asthma, urticaria/angioedema, rhino-conjunctivitis to anaphylactoid shock,^{5,6} and to the cross sensitivity among aspirin (ASA), pyrazoline drugs and nonsteroidal anti-inflammatory drugs, so called

SUMMARY Certain adverse reactions to pyrazoline drugs resemble IgE-mediated hypersensitivity. However, convincing evidence of antigen-antibody interactions is not fully demonstrated. In this study, IgE antibodies specific for 1-phenyl-2,3-dimethyl-3-pyrazoline-5-one have been found in 17 out of 19 serum samples from individuals sensitive to pyrazoline drugs with 4-aminoantipyrine discs by Radio Allergo Sorbent Test (RAST). In contrast, we have not found any positive results from 10 normal donors without sensitivity to pyrazoline drugs after ingestion of metamizol 500 mg/day for 14 days. Therefore, our results provide further evidence in favor of an IgE-dependent mechanism in patients suffering from sensitivity to pyrazoline drugs. The determination of specific IgE antibodies could be used as a serodiagnostics method.

'analgesic sensitivity'.⁷⁻¹⁰ Cross sensitivity between pyrazoline drugs and ASA partially exists.¹¹ Therefore, on one hand, an oral challenge test should be done before using one instead of another. On the other hand, the challenge tests may lead to severe attacks of asthma or urticaria/angioedema. Thus our recent studies focus on establishing serodiagnostic methods helpful for a safe use of analgesics.

MATERIALS AND METHODS

Serum samples and patients' condition

Sera were obtained from 19 patients (7 males and 12 females) having previous positive histories of

sensitivity to pyrazoline drugs. Their ages ranged from 22 to 78 years, with a mean of 39.2 years. Seven patients were documented by oral challenge tests with responsible pyrazoline drugs in our clinic according to a previously described method,¹² 3 of them also had ASA sensitivity documented by oral ASA challenge.¹³ The doses for a positive challenge of propyphenazone or metamizol ranged

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only from 50 mg to 100 mg. The clinical symptoms included asthma 2 (1 with urticaria and another with skin flush), urticaria/angioedema 11, skin eruptions 1, rhino-conjunctivitis with skin flush 1, anaphylactoid shock 4. All the reactions occurred within 4 hours after challenge. Before knowing about sensitivity to pyrazoline drugs, 6 patients complained about possible atopic symptoms which were not the same as those caused by the responsible pyrazoline drugs and only one patient, also being sensitive to ASA, referred to a possibly atopic history in accord with the result of an oral challenge with metamizol. Total IgE concentration was determined by Phadebas IgE RAST (Pharmacia Diagnostic AB, Uppsala, Sweden). Eleven out of 18 samples had total IgE concentration of more than 100 IU/ml. Intracutaneous tests were performed in all 19 patients by injection of 0.02 ml of 0.1% solution of the responsible drug(s). Another 10 serum samples were obtained from normal donors having previous histories of taking pyrazoline drugs without showing sensitivity. They were given metamizol 500 mg/day for 14 days again before their blood was sampled. A serum pool from blood samples of 100 healthy students with total IgE 52 IU/ml was prepared for use as a control.

Reagents

Monoclonal anti-human IgE (BS 17) was a kind gift from Dr. BM Stadler (Bern, Switzerland), sodium iodide I^{125} was obtained from Amer-sham Buchler GmbH and Co. KG (Braunschweig, Germany). Discs (round filter ϕ 6 mm No. 311 660) were supplied by Schleicher and Schuell Co. (Dassel, Germany). The methods of I^{125} -labelling of antibodies for RAST and activation of discs by cyanogen bromide have been described elsewhere.¹⁴ All other chemicals were of analytic grade from Fluka AG (Buchs, Switzerland) and

Sigma Chemical Co. (St. Louis, USA).

Preparation of 4-nitrophenol discs

6-Aminohexanoic acid (200 mg) was solubilized in 30 ml of 0.5 M NaHCO_3 , pH 9.5. Cyanogen bromide-activated discs (3 g) were then added and gently shaken at room temperature for 24 hours. Afterwards, the supernatant was aspirated and the discs were washed with 0.5 M NaHCO_3 , pH 8.4. The remaining reactive groups on discs were blocked with 20 ml of 0.5 M ethanolamine in 0.1 M NaHCO_3 at room temperature for 2 hours. This was followed by washing with distilled water, 0.1 M NaHCO_3 , pH 8.4, and finally with 0.5 M NaHCO_3 , pH 8.4. The treated discs bearing free carboxyl groups are called carboxyl-spacer discs (Fig. 2a).

Carboxyl-spacer discs (1.5 g) were added to a solution containing 4-nitrophenol (100 mg) in 10 ml dioxan. N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (60 mg) was then added and the mixture shaken at room temperature for 24 hours. The supernatant was aspirated and the discs were washed with dioxan, dioxan : H_2O (1:1), dioxan : H_2O (1:10). Finally, The treated discs were called 4-nitrophenol discs (Fig. 2b).

Preparation of 4-aminoantipyrene discs

4-Nitrophenol discs (100 mg) were added to a solution containing 4-amino-antipyrene (5 mg) in 20 ml of 0.5 M NaHCO_3 , pH 8.4. The suspension was rotated end-over-end at 4°C for 24 hours. Afterwards, the supernatant was aspirated and the following steps were the same as the preparation of carboxyl-spacer discs. Finally, the treated discs coupled with 1-phenyl-2,3-dimethyl-3-pyrazoline-5-one were called 4-aminoantipyrene discs (Fig. 2c). They need to be resuspended in incubation buffer (0.1 M sodium phosphate, pH 7.4, containing 0.3%

human serum albumin, 0.1% sodium azide and 0.2% Tween 20). The discs were stored at 4°C until use.

Preparation of salicyloyl-discs and O-methylsalicyloyl-discs

The methods of preparing salicyloyl-discs and O-methylsalicyloyl-discs were described elsewhere.¹⁵ The chemical structure of both discs were presented in Fig. 3.

RAST procedure

50 μ l of serum undiluted were placed onto each disc in plastic tubes (No. 55, 484, Numbrecht, Germany) in duplicate. These were then covered and incubated at room temperature for 3 hours, 2 ml of 0.9% NaCl containing 0.2% Tween 20 were added to all tubes for 10 minutes. This washing procedure was repeated twice. A 50 μ l aliquot of monoclonal anti-human (I^{125}) IgE solution was dispensed onto each disc and the tubes re-incubated overnight. The discs were then washed as before and placed into new tubes. Radioactivity of discs in all tubes and duplicate tubes containing 50 μ l of monoclonal anti-human (I^{125}) IgE was measured in a γ -counter (Berthod, Isernhagen, Germany) for 1 minute and results were expressed as counts per minute (cpm). At least three control serum samples were tested in each assay and the mean value of $\text{cpm} + 3$ standard deviation (mean $\text{CS} + 3$ SD) was calculated. Tested sample values above this were considered positive. (Pharmacia Phadebas RAST 'directions for use' leaflet).

RAST inhibition assay

Briefly, 50 μ l of tested serum was incubated with 50 μ l of either 4-amino-antipyrene in distilled water (+4AA) or just distilled water (-4AA) at room temperature for 1 hour. After centrifugation at 3,000 rpm for 15 minutes 50 μ l was taken out and tested by RAST procedure on 4-aminoantipyrene discs. Percentage inhibition values were calculated using the formula:¹⁶

$$\text{Percentage of inhibition} = 100 - \frac{\text{cpm of } (-4AA) - \text{cpm of control serum}}{\text{cpm of } (+4AA) - \text{cpm of control serum}} \times 100$$

3 SD; the positive rate of RAST was 89.5%. However, only 6 out of 19 patients had weak positive intracutaneous reactions; the positive rate of skin tests was 31.6%.

RESULTS

RAST results are shown in Table 1 and Fig. 4. Comparing 10 serum samples from normal donors after taking metamizol 500 mg/day for 14 days with control serum by using 4-aminoantipyrine discs, ¹²⁵I uptakes of all these tested donors'

samples were under the value of mean CS + 3 SD. In contrast, only 2 samples (No. 16, 17) from the patients with symptoms of anaphylactoid shock compared with control serum had the values less than mean CS + 3 SD while other 17 patients' samples had values more than mean CS +

Oral challenges with responsible pyrazoline drugs and ASA were carried out on 7 patients (Table 1), all of them had positive results using challenge doses of responsible pyrazoline drugs from 50 mg to 100 mg, 3 of them also had positive challenge results with ASA ranging from 25 mg to 500 mg and positive RAST

Table 1. Personal, clinical, oral challenge and RAST data from 19 individuals sensitive to pyrazoline drugs.

Pat	Age (yr)	Sex	Total IgE (IU/ml)	Atopic states	Symtoms	Responsible drugs	Time	Skin tests IC	Dose of oral challenge(mg)	Results of RAST
1	32	F	111	/	A,U	Propyphe Meta	2 yr	+ +	ND ND	+ +
2	33	F	?	/	A,F	Meta	14 d	-	ND	+
3	64	F	43	/	Q	Propyphe	2 yr	-	100	+
4	30	F	383	/	U	Propyphe	6 mo	-	50	+
5	30	M	231	R	U	Propyphe	2 yr	-	ND	+
6	58	M	30	NA	U	Propyphe Phena	1 mo	- -	ND ND	+ +
7	22	M	755	AD	U	Propyphe	4 mo	-	50	+
8	40	M	742	AD	U	Meta	8 mo	+	100	+
9	31	F	17	U,PA	Q	Phena	4 yr	-	ND	+
10	20	F	160	/	Q	Meta	1 yr	-	100*	+
11	35	F	1,000	/	U	Meta	14 d	-	ND	+
12	78	M	400	/	Q	Meta	10 d	+	ND	+
13	30	F	194	CU	U	Meta	1 yr	+	50*	+
14	28	M	100	/	R,C	Meta	1 yr	-	100*	+
15	41	F	17	U,PA	E	Aminophe	5 yr	-	ND	+
16	62	F	24	/	S	Meta	1 yr	-	ND	-
17	30	M	27	/	S	Propyphe	15 yr	-	ND	-
18	48	F	108	/	S	Phena	2 yr	+	ND	+
19	32	F	60	/	S	Meta	6 yr	-	ND	+

Pat = patients' serum number; Time = the period elapsed between the beginning sensitivity of pyrazoline drugs to the blood taken out in this study; A = asthma, U = urticaria, F = flush of skin, E = eruptions, Q = angioedema, R = rhinitis, C = conjunctivitis, S = anaphylactoid shock, AD = atopic dermatitis, NA/PA = novocain/penicillin allergy; Propyphe = propyphenazone, Phena = phenazone, Meta = metamizol, Aminophe = aminophenazone; IC = intracutaneous tests with 0.1% responsible drugs; / = no, ND = not done; + = positive, - = negative; * = with positive ASA challenges.

results with salicyloyl-discs (No. 10, 13, 14), and O-methylsalicyloyl-discs (No. 10, 13), whereas the other four patients were negative with ASA challenges and give negative RAST results with salicyloyl-discs and O-methylsalicyloyl-discs. The challenge results corresponded with the patients' histories. The remaining 12 patients, who did not accept oral challenges, could take ASA without showing sensitivity.

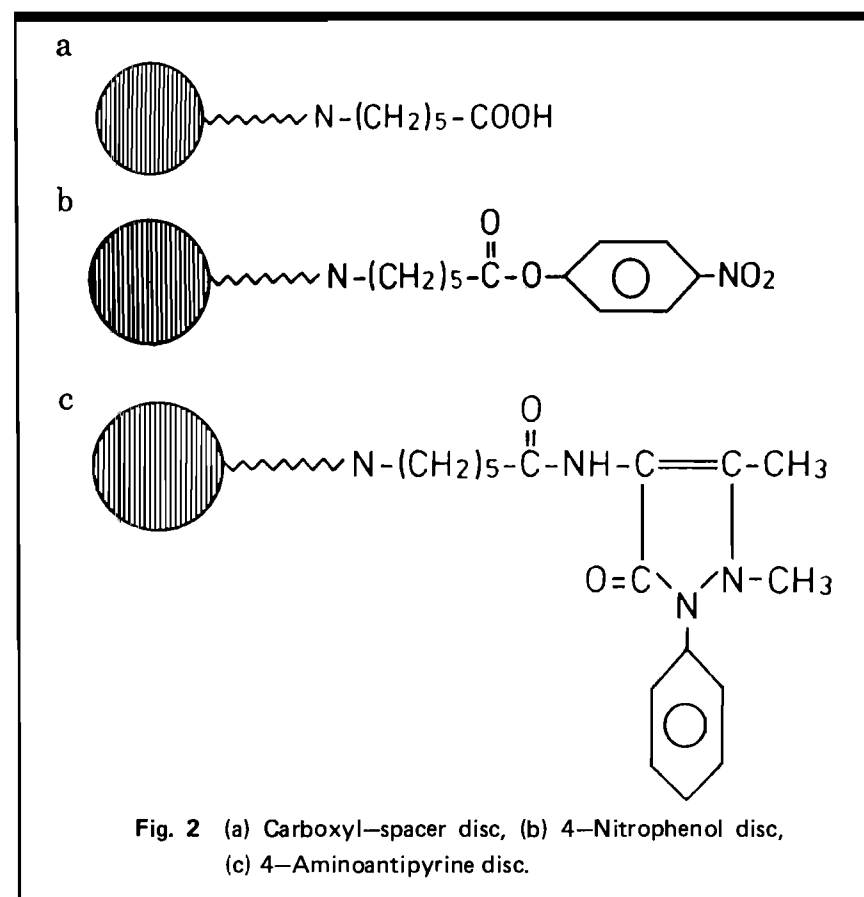
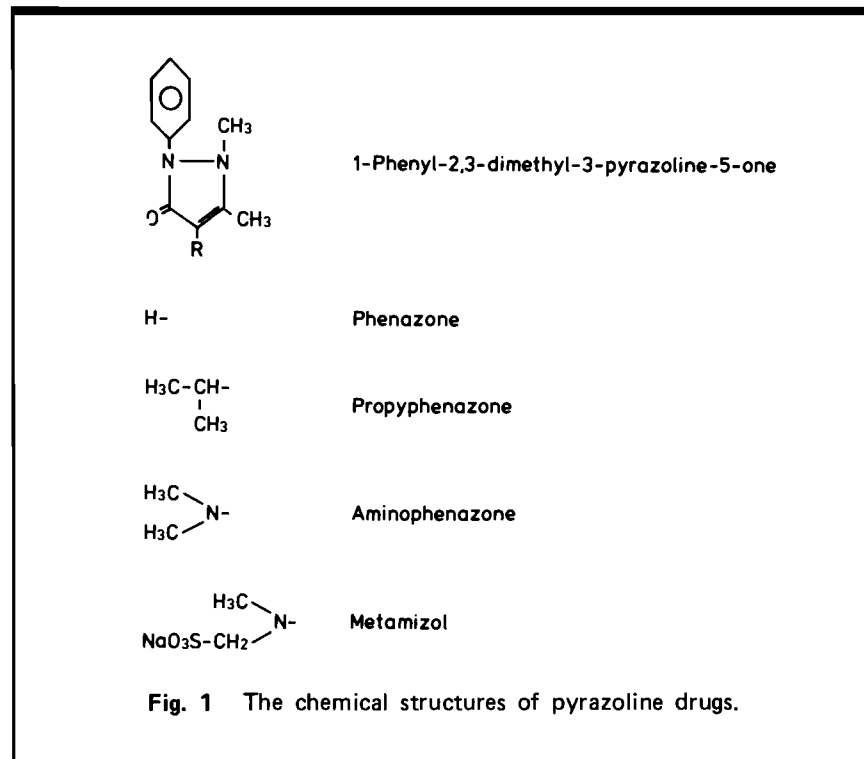
To investigate the specificities of IgE antibodies, further data were obtained from inhibition studies. The patient's serum No. 12 was incubated with 4-aminoantipyrine, then tested by RAST procedure on 4-aminoantipyrine discs. The result of a positive inhibition (>50% inhibition) is shown in Fig. 5.

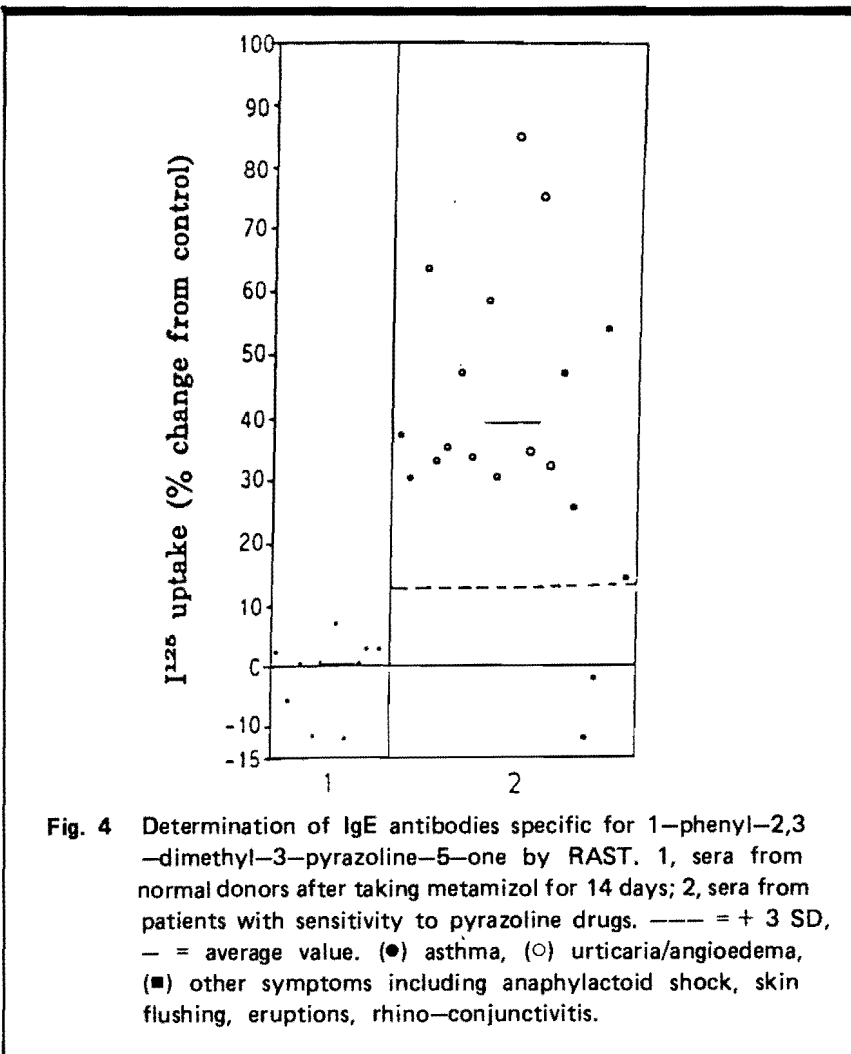
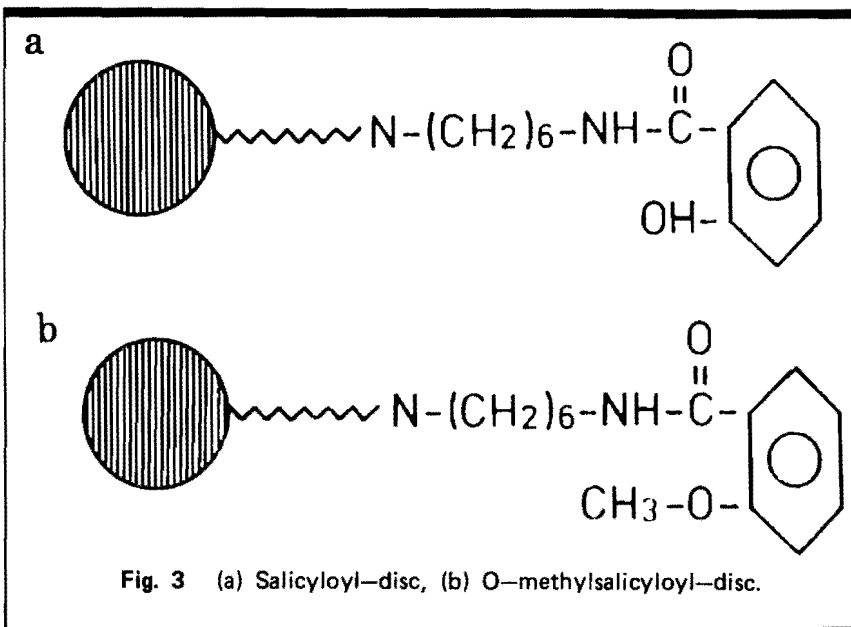
In view of whether the total IgE would affect the results, Two serum samples from atopic patients without sensitivity to pyrazoline drugs having total IgE of 560 IU/ml and 1,748 IU/ml were used in comparison with control serum containing total IgE of 51 IU/ml on 4-aminoantipyrine discs. The percentage change of I^{125} uptakes ranged within $\pm 2\%$.

DISCUSSION

Our study demonstrates the presence of IgE antibodies specific for 1-phenyl-2,3-dimethyl-3-pyrazoline-5-one by RAST procedure on 4-aminoantipyrine discs with a positive rate of 89.5% in 19 patients sensitive to pyrazoline drugs. The pyrazoline drugs are slightly bound to plasma protein and undergo extensive metabolic transformation in man. The most significant primary reactions involve hydroxylation and glucuronidation of the phenyl ring or the side chain (R),¹⁷ thus it was possible to present several types of antibodies in the sensitive patients.

(1). In the patients' sera No. 1-9, 11, 12, 15, 18, 19, the antibodies specific for the epitope of 1-phenyl-2,





3-dimethyl-3-pyrazoline-5-one were detected, since side chains of pyrazoline drugs did not exist in the 4-amino-antipyrine discs. The positive inhibition assay with 4-aminoantipyrine further proved the specificity.

(2). Sera No. 10, 13, 14 from the patients sensitive to pyrazoline drugs and ASA had positive RAST results with 4-aminoantipyrine discs and salicyloyl- or O-methylsalicyloyl-discs. We have previously speculated that ASA-sensitive patients had 'inborn error of metabolism' combined with a particular immunological background.¹⁵ Therefore, the cross reactivity between ASA and pyrazoline drugs is probably caused by the same error of metabolic pathway as follows: The 1-phenyl group on the pyrazoline drugs is ortho-hydroxylated as usual, then O-methylated via O-methyltransferase transferring a methyl group to the ortho-hydroxyphenyl group to be an O-methylphenyl group (Fig. 6). Thus, three types of antibodies specific for corresponding epitopes involving 1-phenyl-2,3-dimethyl-3-pyrazoline-5-one group, ortho-hydroxyphenyl group and O-methylphenyl group could be present simultaneously in those patients. The speculation drawn above was also supported by the fact that ASA-sensitive patients showed the same clinical symptoms from oral challenges with metamizol as with ASA in our patients, and by the data of RAST cross inhibition studies from our previous studies that 4-aminoantipyrine could not neutralize IgE antibodies specific for salicyloyl and O-methylsalicyloyl.¹⁵ But at the present time we do not know why a few primarily ASA-sensitive patients can tolerate pyrazoline drugs without showing sensitivity,¹² whether ASA itself might activate the O-methyltransferase more strongly than pyrazoline drugs.

(3). Sera No. 16, 17 were from the patients with a history of sensitivity to pyrazoline drugs, but we could not detect IgE antibodies speci-

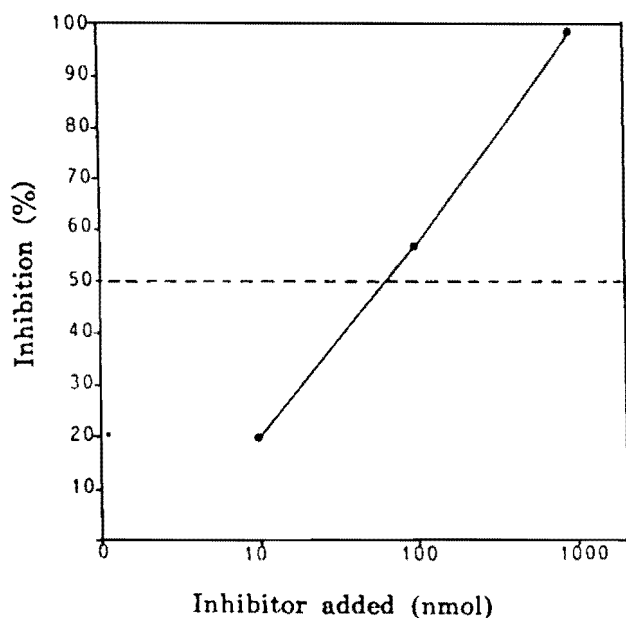


Fig. 5 RAST inhibition assay on 4-aminoantipyrine discs by using patient's serum No. 12 incubated with 4-aminoantipyrine as inhibitor. --- = 50% inhibition.

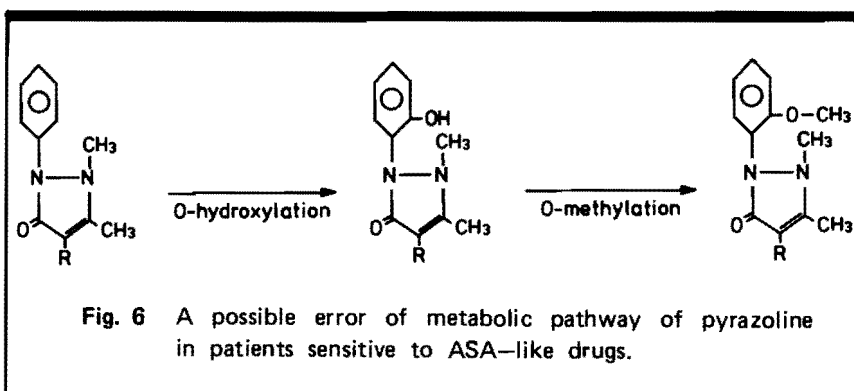


Fig. 6 A possible error of metabolic pathway of pyrazoline in patients sensitive to ASA-like drugs.

fic for 1-phenyl-2,3-dimethyl-3-pyrazoline-5-one. However, two possibilities should be considered: First, serum No. 16 from the patient sensitive to metamizol may have IgE antibodies specific for the side chain;¹⁸ second, sera No. 17 from the patient sensitive to propyphenazone may not have IgE antibodies specific for either 1-phenyl-2,3-dimethyl-3-pyrazoline-5-one or a side chain in the

recent serum sample, since the sensitive reaction occurred 15 years ago before the blood was sampled.

Our data from patients' sera No. 1, 2, 14, demonstrate that different clinical symptoms like asthma, urticaria, flush of skin, rhinitis and conjunctivitis caused by sensitivity to pyrazoline drugs can occur in the same individual. This might be due

to the irregular distribution of sensitized mast cells in these individuals. The fairly low positive rate of skin tests may be related to high lipid/water partition coefficients of pyrazoline drugs and their low ability of binding to protein.¹⁷

In conclusion, we propose that the selective sensitivity to pyrazoline drugs has an immunological background (1), whereas the sensitivity to ASA-like drugs has an immunological background together with 'inborn errors of metabolism' (2). The determination of IgE antibodies specific for 1-phenyl-2,3-dimethyl-3-pyrazoline-5-one by RAST appears an effective and safe serological diagnostic method for sensitivity to pyrazoline drugs.

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