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Impaired Drug Metabolism in Rats Associated with Acute Inflammation: A Possible Assay for Anti-injury Agents (37763)

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The search for new drugs to treat inflammatory disorders is very dependent on the establishment of valid models of fairly chronic inflammation in small animals, especially rodents. In evaluating a potential new drug, it is administered to groups of animals before, during or after establishing the model inflammatory disease. The principal observations are assumed to reflect the effect of the drug on the inflammatory process(es), *i.e.*, its therapeutic activity. Rarely is it considered that the disease may influence the drug action, possibly distorting the therapeutic activity of the compound being studied. As demonstrated below, many forms of experimentally induced local inflammation may have a (systemic) effect on drug disposition and efficacy in the whole animal, primarily by affecting the activity of the drug metabolizing system (DMS)[1] associated with the hepatic endoplasmic reticulum ("microsomes").

Depression of the DMS in rats with established adjuvant arthritis has been described (1–4) but little attention has been given to possible changes in DMS activity in rats following transitory local inflammation. The present observations considerably amplify a preliminary communication (5).

Experimental Methods. Inbred male Lewis (Microbiological Associates, Walkersville, MD) and outbred male HLW Wistar rats (Hilltop Lab. Animals, Chatsworth, CA) weighing 150–250 g were caged in groups of 4 on sawdust and permitted unlimited access to food and water. After adrenalectomy (via dorsal incision) they were given 0.9% NaCl to drink. Light ether anesthesia was used for foot immersion, surgical procedures, withdrawing blood, etc.

Aminopyrine and cyclophosphamide metabolism by liver preparations (fortified homogenates or microsomes) was measured as described (3). Drug metabolism *in vivo* was measured by the duration of the sleeping time after administering sodium hexobarbital (150 mg/kg) or trichloroethanol (200 mg/kg) ip. Trichloroethanol (TCE) was formulated for injection by diluting 1.3 ml TCE with 3.5 ml propylene glycol and 95 ml saline and injecting 1 ml/100 g body weight.

Arthritogenic adjuvants and nonarthritogenic "pseudoadjuvants" = either 10 mg/ml delipidated heat-killed *M. tb.* (human) or 4 mg/ml BCG cell walls dispersed in various oily vehicles (3). Other irritants were those commercially available and are described in the Results section. After a single oral dose of xanthotoxin (XT) (Sigma, St. Louis, MO), rats were exposed to direct sunshine for at least 4 hr; with appropriate controls receiving only XT or sunshine. Drugs were administered po except where noted, in 1% gum arabic. Foot swelling was measured with a micrometer screw gauge.

Results. Unless specified, results described are those principally obtained with Wistar (HLW) rats. Depression of DMS was primarily monitored by prolongation of the

[1] Abbreviations used: DMS = drug metabolizing system (associated with rat liver microsomes); 6-MP = 6-mercaptopurine; XT = xanthotoxin (methoxsalen, 8-methoxypsoralen); *M. tb.* = *Mycobacterium tuberculosis*; NBP = 4-*p*-nitrobenzylpyridine; CPA = cyclophosphamide; R-760 = Flazalone; FA = Freund's adjuvant (*M. tb.* in mineral oil); TCE = 2,2,2-trichloroethanol; BCG = *M. bovis* (B. Calmette Guérin).

sleep time after injecting ip hexobarbital (150 mg/kg). Normal animals slept for 25 ± 4 min ($n = 32$) with this dose.

Effect of adjuvants and "pseudoadjuvants". Adjuvants initiating arthritis in all four limbs after 14 days induced rapid foot swelling when injected (50 μl) into a rear paw. Pseudoadjuvants which were not arthritogenic also caused acute swelling which subsided entirely within 2 wk or less. After 2 wk, only animals with arthritis had a depressed DMS. However, both types of adjuvants caused significant extension of hexobarbital sleeping times within 2 days of their inoculation. This effect of the pseudoadjuvant declined as the acute inflammation subsided and almost normal sleeping times were obtained by Day 7 after inoculation.

Effect of site of adjuvant inoculation. Adjuvants and pseudoadjuvants (50 μl) administered at the base of the tail elicited similar changes in the DMS as when inoculated into a rear paw, but signs of acute inflammation were usually minimal. Tail-injected adjuvants elicited all the signs of arthritis.

Adjuvants normally inducing arthritis (when inoculated in the tail or rear paw) occasionally did so when given ip and though causing a diffuse irritation throughout the peritoneal cavity, did not significantly extend the sleeping times at 48 hr. Only those animals developing subsequent arthritis displayed extended sleeping times by Day 14.

Inoculating an arthritogenic adjuvant (*M. tb.* in mineral oil, F A) into one ear caused a local necrosis and initiated arthritis 14 days later in all four limbs and tail in Lewis and Wistar rats, but had *no* significant effect on the DMS, as judged by hexobarbital sleeping times 1–3 days later. Similarly, arthritogenic adjuvants injected in a front paw caused acute inflammation but had surprisingly little effect on the DMS. However, when arthritic, these ear- or front paw-inoculated animals had elevated hexobarbital sleep times.

Effect of quantity of adjuvant. The above results were obtained after inoculating full-strength adjuvants or pseudoadjuvants, *i.e.*, 500 μg *M. tb.* in appropriate vehicle. Injecting a one-tenth strength arthritogenic adjuvant (*i.e.* 50 μg *M. tb.* in 50 μl mineral oil) in the tail, consistently caused arthritis at 14 days but did not lengthen the sleep time at 48 hr. The small volumes of full-strength adjuvant that could be injected into the inguinal nodes (preexposed under anesthesia) likewise successfully initiated the arthritis but had no effect on the sleeping time determined 48 hr later.

Effect of other irritants and inflammagenic stimuli (Table I). Three classes of irritants/stimuli could be distinguished according to their effect on the DMS:

1. "Bland" *i.e.*, causing inflammation and paw swelling but not affecting sleep times 24 or 48 hr later (see Table IA);

2. Acute, causing (usually transitory) inflammation and lengthened sleep times over the interval 24–72 hr thereafter, with subsequent recovery of DMS by the seventh or eighth day (Table IB);

3. Chronic, causing inflammation which subsided slowly and prolonged depression of DMS (Table IC).

Examples of bland irritants were polyethylene glycol (0.1 ml, mol wt 380–420) injected in both paws, immersion (of one rear paw only) in water at 45° for 30 min or at 56° for 30 sec, application of solid CO_2 for 30 sec, exposure to sunshine (10 AM–2 PM) for three consecutive low-smog days, injection of both paws with serotonin (100 μg base as oxalate salt in 50 μl saline) or brewer's yeast (0.1 ml 2.5% saline suspension) or 1 mg/foot of a highly edemagenic type of carrageenan (Seakem, CT) or 100 mg/foot of kaolin (Baker, Phillipsburg, NJ) suspended in saline.

Acute depressants of the DMS included laparotomy, ip injection of kaolin (100 mg), foot injection of some samples of carrageenan with low edemagenic activity (*e.g.*, 1 mg calcium salt, Sigma, St. Louis, MO); 50 μl turpentine; 10 μl croton oil (Schuchardt, Munich, DBR); a variety of pseudoadjuvants (50 μl) constituted with whole *M. tb.* or BCG cell walls dispersed in nonarthritogenic oily vehicles (methyl oleate, hexane, oleyl alcohol); another "family" of pseudoadjuvants constituted with nonarthritogenic bacteria (*e.g., C. hoffmannii,* a strain of *M. Phlei*) dispersed in vehicles which, in

TABLE I. Effect of Acute Inflammation on Hexobarbital (HB) Metabolism (Sleep Time) in Rats.[a]

Rat strain	Irritant	Injection site	Local inflammation	Sleep time ± SEM			No. animals
				Day 1	Day 2	Day 3	
A.							
Wistar	Control	—	—	20 ± 14	20 ± 14	20 ± 14	20
	N-Carrageenan	Foot/tail	++	36 ± 12	23 ± 7	40 ± 0	13
	M.tb. in saline	Foot/tail	—	—	19 ± 6		6
	Mineral oil	Foot/tail	—	—	19 ± 11	25 ± 9	4
	Kaolin	Foot/flank/tail	—	—	—	25 ± 6	9
	Polyethylene glycol	Foot	+	—	17 ± 17	—	2
	Hot H_2O	Foot	+	—	14 ± 5	—	5
	CO_2 (dry ice)	Foot	+	—	23 ± 3	—	3
	Sunshine, 12 hr	Whole body	+	—	10 ± 8	—	6
	Serotonin	Foot	+	—	26 ± 5	—	7
	Brewer's yeast	Foot	+	—	18 ± 6	—	5
HLA (F344xW) F_1 hybrids	Control	None		—	26 ± 7	—	5
	Viable Wistar spleen cells	Foot	—	—	31 ± 10	—	3
	Heat-killed Wistar spleen cells	Foot	—	—	39 ± 3	—	3
	Viable Lewis spleen cells	Foot	—	—	15 ± 15	—	2
B.							
Wistar	Control	—	—	20 ± 14	20 ± 14	20 ± 14	20
	Laparotomy	—	—	74 ± 4	—	81 ± 8	10
	Calcium Carrageenan	Foot	+	—	62 ± 10	—	4
	Kaolin	ip	—	—	48 ± 5	—	8
	Turpentine	Foot	+++	45 ± 11	67 ± 8	54 ± 9	15
	Croton oil	Foot	++++	39 ± 13	57 ± 9	83 ± 1	8
	Nonarthritic adjuvants	Foot/tail	++	Days 1–4		90 ± 8	26
C.							
Wistar	Control	—	—	—	—	28 ± 15	49
	Arthritic adjuvant	Foot/tail	+++	Days 1–4		75 ± 3	70
	Sunlight and xanthotoxin	Oral/whole body	+++	Day 2 postexposure to sunlight			
				88 ± 11			4
Buffalo	Control	—	—	Days 1–4		25 ± 5	10
	Arthritic adjuvant	Foot/tail/node	+++	Days 1–4		123 ± 12	12
Lewis	Control	—		Days 1–4		25 ± 4	16
	Arthritic adjuvant	Foot/tail	+++	Days 1–4		65 ± 3	19

[a] Irritants (see text) administered on Day 0 and sleep time was determined 1, 2, 3 and 4 days thereafter (HB, 150 mg/kg, ip).

combination with *M. tb.*, are arthritogenic, *e.g.*, dodecane, hexadecane, triolein, butyl oleate, mineral oils. All these materials caused local inflammation with foot swelling of two or more days' duration.

Chronic depressants of the DMS were arthritogenic adjuvants (*e.g., M. tb.* in normal paraffins $> C_2$) and exposure to uv light (sunlight) after a single oral dose of xanthotoxin (20 to 50 mg/rat). Constituents of these noxious inflammagens, *i.e., M. tb.* (administered in saline) or mineral oil alone, XT or sunshine alone, administered singly caused minimal inflammation and were bland irritants; in contrast to *M. tb.* with oil or XT plus light which caused chronic tissue injury and a long-lasting depression of the DMS (up to 8 wk with *M. tb.*; over 18 days with XT and uv).

Establishment of a profound immunological reaction with gross lymphadenopathy in the draining popliteal and elbow lymph nodes (graft vs host disease) by inoculating 300×10^6 parental strain (Wistar) splenic lymphocytes in each of the four paws of tolerant Wistar $\times$ Fisher F_1 hybrid rats (6) had no effect on the liver DMS 2 or 7 days after cell grafting. Neither was the DMS depressed when viable spleen cells from Fisher, Lewis or Buffalo rats were injected into the rear paws of Wistar rats.

In vitro *studies.* Aminopyrine demethylation and cyclophosphamide (CPA) hydroxylation in fortified rat liver homogenates was determined by measuring the yield of formaldehyde and (NBP-reactive) alkylating metabolites, respectively (3). Adjuvants, pseudoadjuvants and turpentine injected 2 days previously into the tail and rear paw of the liver donor animals significantly depressed the DMS. Prior injection of (edemagenic) carrageenan selectively altered the DMS activity (Table II), having no effect on aminopyrine metabolism but depressing CPA activation.

Selective effect of acute irritants on hydroxylation. Many animals with *established* arthritis show prolonged sleep times with trichloroethanol (TCE) as well as with hexobarbital (3). TCE is not hydroxylated (like hexobarbital) but forms a glucuronide conjugate (7). TCE sleep times were much closer to normal in prearthritic animals and 2 days following a pseudoadjuvant (*M. tb.* in hexane) ip kaolin or croton oil (in foot), when the same groups of animals had significantly extended hexobarbital sleep times (Table I). These findings suggest that different facets of the DMS may be affected to different degrees by short-term inflammation.

Effect of adrenalectomy and splenectomy. A pronounced perisplenitis develops in animals with adjuvant arthritis 16 days after the adjuvant inoculation. Animals splenectomized 3 days before inoculation with either an arthritogenic (*M. tb.* in mineral oil) or nonarthritogenic (*M. tb.* in hexane) adjuvants showed the same prolonged sleep time at 48 hr as both sham-operated and normal controls inoculated with these adjuvants.

TABLE II. *In Vitro* Metabolism of Cyclophosphamide (CPA) and Aminopyrine (AP) by Liver Preparations from Wistar Rats Suffering Acute Inflammation.

Irritant injected	Local inflammation	% Metabolite formation[a]		No. animals
		CPA	AP	
Control	—	100	100	20
Nonarthritic adjuvants	+	45 ± 6	45 ± 10	13
Arthritic adjuvants	++	46 ± 5	58 ± 5	19
Turpentine	+++	47 ± 1	48 ± 3	4
N-Carrageenan	+	56 ± 9	105 ± 11	10

[a] Determined 2 days after inducing local inflammation: figures are percentages of concurrent controls, run simultaneously (± SD). Average values in these controls were 5.3 ± 3.5 nmoles of alkylating metabolites (calculated as equivalents of mechloreth amine)/15 min/mg protein ($n = 43$) from CPA, and 80 ± 74 nmoles formaldehyde/15 min/mg protein ($n = 28$) from AP.

TABLE III. Effect of Some Antiarthritic Drugs on Rapid Impairment of Hexobarbital Metabolism in Lewis (L) or Wistar (W) Rats Injected with Freund's Adjuvant (FA)[a]

Rat strain	Drug[b]	mg/kg day	*P* value	Sleep time (min)	No. of determinations	Foot swelling (10^{-3} cm) Day 1–3	Foot swelling (10^{-3} cm) Day 0–17
W	No drug (no FA)[c]		—	22 ± 3	25	—	21 ± 6
L	No drug (no FA)[c]		—	27 ± 3	14	—	45 ± 10
W	No drug		—	65 ± 4	30	318 ± 23	521 ± 40
L	No drug		—	67 ± 3	14	385 ± 19	416 ± 63
W	Dexamethasone	0.5	<0.001	12 ± 6	6	175 ± 47	363 ± 97
L	Prednisolone	10.0	NS	62 ± 4	8	287 ± 25	408 ± 94
L	Corticosterone (sc)[d]	50.0	<0.001	46 ± 3	8	130 ± 9.5	296 ± 91
W/L	Melengesterol acetate	100	<0.001	49 ± 3	12	237 ± 34	395 ± 62
W/L	Azathioprine	60	<0.05	45 ± 10	10	205 ± 52	218 ± 79
W/L	6-MP	50	<0.001	42 ± 4	18	345 ± 26	349 ± 51
W	Cycloleucine	50	NS	78 ± 6	5	288 ± 23	67 ± 25
L	ICI-43,823	200	NS	103 ± 4	7	278 ± 27	198 ± 82
W/L	Indomethacin	1	NS	83 ± 8	10	253 ± 24	448 ± 86
W	Flazalone (R-760)	100	NS	65 ± 6	5	155 ± 18	230 ± 92
W	Cyclophosphamide	30	NS	69 ± 11	11	174 ± 26	98 ± 40
L	Chlorambucil	15	NS	67 ± 2	8	307 ± 49	320 ± 136
W	Azauridine triacetate	200	NS	87 ± 7	6	278 ± 31	307 ± 95
W	Penicillamine[e]	200	>0.05	55 ± 6	8	323 ± 17	672 ± 71
W	Na Aurothiomalate (im)	25	NS	57 ± 8	8	151 ± 38	456 ± 12
W	Cytosine arabinoside[e] (ip)	50	NS	65 ± 3	6	260 ± 82	395 ± 93
W/L	Methotrexate	10	>0.05	55 ± 5	13	235 ± 66	All died
W	ENDO-3638	100	>0.05	54 ± 6	8	256 ± 64	371 ± 102
L	Phenylbutazone	10	NS	66 ± 6	6	283 ± 20	392 ± 79
L	Chloroquine diphosphate	100	NS	66 ± 4	8	452 ± 20	474 ± 81

[a]Drugs administered daily for 5 days only (see text). Sleeping times after 150 mg/kg Na hexobarbital determined 2 and 3 days after FA.

[b]po except as noted.

[c]Not injected with adjuvant-normal controls. All other groups of animals received FA.

[d]Administered subcutaneously in 0.2 ml arachis oil.

[e]Given in 2 divided doses/day, all other drugs given once daily.

Animals adrenalectomized 5 or 9 days before inoculation with turpentine or a pseudoadjuvant (*M. tb.* in hexane) appeared to have the same (or even lengthened) sleeping times as normal (intact) animals inoculated with the same acute irritants. However on being challenged with these irritants a high mortality ensued (11/15 in one group) among otherwise perfectly healthy adrenalectomized animals. Although the numbers of survivors were too small for complete confidence in the data, the (prolonged) sleeping times were apparently not reduced by prior adrenalectomy.

Effect of drugs (*Table III*). Animals were injected with *M. tb.* in mineral oil (FA) in one rear paw on Day 0 and drugged once daily for only 5 days (Days $-1 \rightarrow +3$), their hexobarbital sleep times were determined 2 and 3 days later and the signs of arthritis were scored 14 days later. Parallel groups of animals received the drugs but no FA. Dexamethasone, corticosterone, azathioprine and 6-MP each protected the animals from the effect of FA on the DMS. Only dexamethasone shortened the sleep time in normal animals (no adjuvant). This effect of the thiopurines was noteworthy since they showed little anti-inflammatory activity and were only modestly antiarthritic. Other immunosuppressant drugs (*e.g.*, cycloleucine, cyclophosphamide, azauridine triacetate) suppressed arthritis development but did not prevent the "recognition" of FA which leads to altered drug metabolism. Nonsteroid drugs which powerfully suppress acute inflamma-

tion and paw swelling (*e.g.,* R-760, indomethacin) had little or no effect in preventing the DMS depression.

The sleep times of animals inoculated with a nonarthritogenic adjuvant (*M. tb.* in hexane) 2 and 3 days previously were also reduced by 6MP ($P < 0.01$) and melengesterol acetate ($P < 0.01$).

Discussion. Other rapid systemic responses associated with acute and chronic local inflammation include reduction in serum albumin (8, 9), rise in circulating α_2-glycoproteins (8) and fibrinogen (10); all reflecting altered biosynthetic activities of the liver.

The chief characteristics of this lesion in hepatic drug metabolism induced by distal inflammation are (a) its rapid onset, (b) its brief duration unless inflammation is unduly prolonged, and (c) its sensitivity to certain drugs. Physical stimuli applied locally to a foot such as osmolar shock, heat, cold and (uv) light though causing measurable edema had apparently little or no effect on the liver. Certain chemical inflammagens such as locally applied serotonin and yeast also had no effect on liver metabolsim as evidenced by normal hexobarbital "sleep times." Carageenan samples used for eliciting acute paw edemas in screening new compounds for anti-inflammatory activity also fell into this category of bland inflammagens. However other less edemagenic samples of carrageenan did induce alterations in drug metabolism and provided "exceptions to the rule" that severity of the DMS dysfunction reflected severity of the local inflammation. In general, powerful chemical irritants (*e.g.,* turpentine and adjuvants) elicited both a severe local inflammation and sufficient alteration in the drug-metabolizing capacity of the liver to raise the question of the effect of the local "disease" having a systemic effect on drug distribution and metabolism. Even some mild irritants (*e.g.,* kaolin) had this pathopharmacodynamic effect.

Neither the spleen nor the adrenals had an obligatory role in triggering altered DMS activity as a consequence of local inflammation. This contrasts with the effect of adrenalectomy in ablating the production of α_2-glycoproteins, in response to turpentine (11) or croton oil (8). However, adrenalectomy has little effect on the fibrinogen response to inflammation (8) or certain biological activities of leukocyte extracts (12).

These findings have at least two implications for future drug studies:

1. The effect of the inflammation (elicited with an arthritogenic adjuvant) on the metabolism of a drug, being investigated for anti-arthritic activity, might be minimized by inoculating the adjuvant in a forepaw or ear where the local injury is much more restricted. According to this protocol and until systemic inflammatory disease develops, the DMS functions more normally and the risk of a drug being prematurely discarded, due to overt toxicity (impaired metabolism), is thereby reduced.

2. The remarkable effects of two thiopurines and dexamethasone in preventing DMS depression suggest that these drugs might prevent certain types of tissue injury, at distal sites, over and beyond their activities in modulating local tissue damage. If this property of these drugs can be established independently, then measurements of sleep times after adjuvant inoculation in a rear paw (or the tail) could perhaps be used to search for other agents of this type (with due checks for DMS induction in animals not receiving an adjuvant). Present anti-inflammatory assays may be rather inadequate for recognizing the possible "worth" of 6-MP and azathioprine as anti-injury agents, though sufficient for detecting that of corticosteroids.

Neither 6-MP (50 mg/kg/day) nor azathioprine (60 mg/kg/day) protected female rats from indomethacin-induced ulcers, under the conditions that prednisolone or spironolactone (100 mg/kg, b.i.d.) did so, indicating that these thiopurines were not "catatoxic" (13, 14) at reasonable doses. Their putative anti-injury activity is therefore unlikely to be due to a selective induction of drug metabolism.

Summary. 1. Many acute irritants, e.g., turpentine, mimicked Freund's adjuvant (FA) in causing rapid depression of hexobarbital and cyclophosphamide metabolism in rats *in vivo.*

2. Metabolism of cyclophosphamide and

aminopyrine *in vitro* by liver homogenates was often subnormal if liver donor animals had been acutely inflamed (rear paw, tail) 48 hr previously.

3. Three classes of irritants/inflammagenic stimuli were distinguished by their ef fects on liver drug metabolism.

4. Carrageenan as routinely used for anti-inflammatory screening had a peculiar, selective effect on drug metabolism.

5. Drug metabolism was distinctly subnormal in animals inoculated with FA (rear paw, tail) at all stages prior to developing arthritis.

6. Drug metabolism was almost normal in prearthritic animals when FA was inoculated ip in a front paw or ear. Ear inoculations induced arthritis consistently in Lewis and Wistar rats.

7. Several drugs which prevented development of arthritis failed to normalize drug metabolism in the 4-day period after inoculating FA in rear paw or tail.

8. 6-MP and dexamethasone prevented the acute depression of drug metabolism and may be prototypes of a new class of anti-injury agents.

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1. Morton, D. M., and Chatfield, D. H., Biochem. Pharmacol. **19**, 473 (1970).

2. Whitehouse, M. W., and Beck, F. J., Drug Metab. Disposition (ASPET) **1**, 251 (1973).

3. Beck, F. J., and Whitehouse, M. W., Biochem. Pharmacol., in press.

4. Zak, S., Honc, F., and Lukas, G., Int. Congr. Pharmacol., 5th, San Francisco, 1973. Abstr. No. 1549 (p. 259).

5. Whitehouse, M. W., Proc. SBFTE Symp. Inflammation, Ribeirao Preto, S.P. (Brazil), May, 1973, in press.

6. Swingle, K. F., Grant, T. J., and Valle, P. M., Proc. Soc. Exp. Biol. Med. **142**, 1329 (1973).

7. Williams, R. T., "Detoxication Mechanisms," 2nd ed. p. 56. Wiley, New York (1959).

8. Glenn, E. M., Bowman, B. J., and Koslowske, T. C., Biochem. Pharmacol. Suppl. p. 27 (1968).

9. Coppi, G., and Bernardi, G., Arzneim.-Forsch. **22**, 237 (1972).

10. Glenn, E. M., Biochem. Pharmacol. **18**, 317 (1969).

11. Weimer, H. E., and Benjamin, D. C., Proc. Soc. Exp. Biol. Med. **122**, 1112 (1966).

12. Kampschmidt, R. F., Upchurch, H. F., and Pulliam, L. A., Proc. Soc. Exp. Biol. Med. **143**, 279 (1973).

13. Selye, H., Exp. Med. Surg. **28**, 169 (1970).

14. Selye, H., J. Pharm. Sci. **60**, 1 (1971).

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