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Inhibition of Antigen–Antibody Reactions *in Vitro* by Nonsteroidal Anti-Inflammatory Agents (32634)

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Although the etiology of rheumatoid arthritis and other connective tissue diseases remains unknown, an increasing body of evidence suggests that these may be autoimmune diseases (1). Even though no specific antigen capable of initiating rheumatoid arthritis has been isolated, the possibility exists that the disease could result from an interaction of an altered gamma globulin with rheumatoid factor on synovial and connective tissue cells. Furthermore, Rawson and Torralba demonstrated that injections of complexes of heat-aggregated gamma globulin and rheumatoid factors into normal rabbit joints induce lesions similar to those observed in rheumatoid arthritis in humans (2). Thus, agents useful in treating rheumatoid arthritis might exert their therapeutic effects, at least in part, by preventing the interaction of these immunologic species.

Investigations with salicylates indicate that these compounds affect immunologic phenomena (3). Friend (4), using *in vitro* anti-egg albumin and antipolysaccharide systems with appropriate antigens, observed that sodium salicylate and its analogs inhibit antigen–antibody precipitation reactions. In this study we compare other nonsteroidal anti-inflammatory drugs in their ability to inhibit similar antigen–antibody reactions.

Materials and Methods. Four albino rabbits (2–4 kg) were injected twice weekly for 3 weeks with 1.0% egg albumin (EA) in isotonic saline. Initially each rabbit received 2.0 ml of EA solution intravenously followed by 1.0 ml of a 1:1 suspension of EA solution and corn oil subcutaneously in the foot pads. Subsequent intravenous doses were 1.0 ml of EA solution so that each animal received a total of 75 mg protein. Seven days after the last injection the animals were anesthetized (25 mg/kg pentobarbital Na, iv) and bled by catheterization of the carotid arteries. Blood was pooled and allowed to clot overnight in the refrigerator before centrifuging at 5°C for 15 min at 1470 *g* in a RC-2 Sorvall centrifuge. A gamma globulin fraction was prepared from the pooled sera as outlined below.

After precipitation at 33.3% saturation with ammonium sulfate, pH 7.0, the gamma globulin (Anti-EA) fraction was dissolved in 0.005 *M* sodium phosphate buffer, pH 7.0, and dialyzed in the same buffer. The dialyzate (about 60 ml) was divided into 5-ml aliquots and frozen until used. Each milliliter contained 19.1 mg protein. Maximum precipitation was obtained after incubation at 37°C for 1 hour using 0.5 ml Anti-EA mixed with 0.5 ml of a 1:100 dilution of 1.0% EA solution.

In inhibition studies, 0.5 ml of a 1:100 dilution of 1.0% EA was added to 0.5 ml of Anti-EA followed immediately by 2.0 ml

TABLE I. Inhibition of Immunologic Reactions *in Vitro* by Anti-Inflammatory Drugs.

Drug	Conc. (mg/ml)	Absorbance ± SEM	Inhibition %	Plasma levels[b] (mg/ml)
Saline	— (13)[a]	0.181 ± 0.003	00.0	—
Aminopyrine	40.0 (2)	0.099	45.3	—
Indomethacin	3.3 (2)	0.153	15.5	0.0074 (8)
	16.7 (7)	0.030 ± 0.004	83.4	
Phenylbutazone	3.3 (2)	0.171	5.5	0.02–0.08 (6, 7)
	16.7 (11)	0.063 ± 0.012	65.2	
Salicylate, Na	4.0 (2)	0.159	12.2	0.25–0.50 (9)
	8.0 (2)	0.126	30.4	
	16.0 (2)	0.063	65.2	
	80.0 (9)	0.021 ± 0.002	88.4	

[a] Values in parentheses indicate number of determinations.

[b] Reported plasma levels. Values in parentheses are references.

of drug solution. After incubation at 37°C for 1 hour turbidity measurements were quantitated at 600 mμ in a Beckman model B Spectrophotometer. Control samples contained 0.5 ml of Anti-EA, 0.5 ml EA, and 2.5 ml saline. All drugs were dissolved in isotonic saline and adjusted to pH 7.4. A 1.0% EA solution in 0.067 *M* sodium phosphate buffered saline, pH 7.4, served as stock antigen. All solutions were prepared daily.

Results and Discussion. Data in Table I compare the efficacy of phenylbutazone, indomethacin, aminopyrine, and sodium salicylate in inhibiting EA–Anti-EA reactions. At concentrations of 16.7 and 3.3 mg/ml, indomethacin is more potent than phenylbutazone and by comparison in log dose-response curves with sodium salicylate at 4.0, 16.0, and 80 mg/ml, both are more potent than the latter. A higher concentration of aminopyrine was needed to attenuate the EA–Anti-EA reaction than any of the drugs tested. We were unable to test many other anti-inflammatory agents because of solubility difficulties at pH 7.4.

Although we were able to demonstrate more inhibition of the EA–Anti-EA reaction with comparable concentrations of sodium salicylate, our findings generally agree with those of Friend (4). Thus we observed 88.55% inhibition with 80 mg/ml of sodium salicylate and Friend obtained 54.4% inhibition.

The order of inhibitory activity with tested anti-inflammatory agents, viz. indomethacin > phenylbutazone > salicylate > aminopyrine, is in agreement with data obtained in other laboratory models used to detect anti-inflammatory activity (5). However, the *in vitro* concentrations required to inhibit this antigen–antibody reaction far exceed known plasma levels of these agents in humans (Table I), with the exception of salicylate. Doubt then arises as to whether these drugs exert their pharmacologic action in humans by inhibiting reactions between rheumatoid factors and altered tissue components at cell surfaces thus diminishing subsequent cell damage.

Summary. Aminopyrine, indomethacin, phenylbutazone, and sodium salicylate were observed to inhibit precipitation reactions on incubation of egg albumin with rabbit anti-egg albumin. Concentrations of anti-inflammatory agents needed to produce inhibition of antigen–antibody reactions *in vitro* far exceeded reported plasma levels in humans.

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Characteristics of a Subcellular System from *Cellvibrio gilvus* for the Incorporation of Amino Acids into Protein (32635)

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The bacterium *Cellvibrio gilvus* can be induced to synthesize extracellular cellulases—four enzymes which are all specific for both the second and the third glucosidic bonds from the nonreducing end of the cellulose molecule (1)—when grown in a medium containing cellulose. Investigations of the location of these cellulases within the cell have revealed that the membrane bound ribosomes contain an unusually high enzymic activity (2). In order to study the location of cellulase synthesis by this organism, it was necessary to prepare and characterize a cell-free amino-acid incorporating system from it.

This report details the properties of such an amino acid incorporating system, giving the effects of ribosome concentration, pH, temperature, pH 5 fraction, and magnesium ions. Furthermore, the effects of transfer RNA, polyuridylic acid, antibiotics, polyamines, deoxyribonuclease and ribonuclease are presented. In the present investigation, we have not attempted to characterize the proteins which were synthesized during the course of amino acid incorporation.

Materials and Methods.[1] *Cell-free preparations. Cellvibrio gilvus,* strain 11, obtained from Dr. K. W. King, was grown at 30°C with strong aeration in the culture medium of Hulcher and King (3) with the following modifications: 0.25 gm of $(NH_4)_2SO_4$ was substituted for an equal weight of $NaNO_3$ per liter; 2 drops of antifoam (Nalco 71-D5, Nalco Chemical Co., Chicago, Ill.) were added per liter; the pH was adjusted to 6.6 with HCl. Cell growth was measured using a Bausch and Lomb Spectronic 20 colorimeter with 0.5 inch test tubes at 540 mμ. Cells were harvested during early log phase of growth, when the absorbancy was between 0.3 and 0.4. The wet weight yield of cells was about 0.8 gm/liter.

Cells were isolated by centrifugation in a refrigerated centrifuge and all following steps were performed in the cold. After washing the cells three times with 0.02 *M* Tris–HCl buffer, pH 7.8, containing 0.009 *M* $Mg(Ac)_2$, 0.07 *M* KCl, 0.006 *M* β-mercaptoethanol (Matheson, Coleman, and Bell), referred to hereafter as "buffer", the cells were suspended in buffer (3 gm of packed cells per 10 ml of buffer) and sonicated for 45 sec at maximum output on a Model DF 101 Raytheon Sonic Oscillator, Waltham, Mass. The resulting material was centrifuged twice at 27,000 *g* for 15 min and the supernatant was spun for 1 hour at 100,000 *g*. The top three-fourths of resulting supernatant were removed by aspiration, brought to pH 5 by the dropwise addition of 1 *N* acetic acid and the resulting precipitate was collected by centrifugation and dissolved in buffer, which was brought to pH 7.8, and used in the cell-free system. This is referred to as the pH 5 fraction. The 100,000 *g* precipitate, which is referred to as the "ribosomes," was washed with buffer, recentrifuged and suspended in buffer for immediate use in the cell-free system.

Preparation of transfer-ribonucleic acid

[1] Tris, tris(hydroxymethyl)aminomethane; poly-U, polyuridylic acid; tRNA, transfer ribonucleic acid; ATP, adenosine 5′triphosphate; GTP, guanosine 5′-triphosphate; UTP, uridine 5′-triphosphate; CTP, cytidine 5′-triphosphate; PEP, phosphoenolpyruvate; PK, pyruvate kinase; TCA, trichloroacetic acid; $Mg(Ac)_2$, magnesium acetate.