fractions of bile and whole gallbladder bile suggest that the lecithin and cholesterol are closely associated and remain so even when the bile salts are removed. Whatever the manner of combination within the micelle, both fatty acids of the lecithin molecule probably contribute to the solubilization of the cholesterol since preliminary studies from this laboratory have shown that lysolecithin solubilized only about one half the amount of cholesterol solubilized by lecithin (10).

Summary. Model synthetic solutions were used to study cholesterol solubilization by the 6 conjugated bile salts found in human bile individually and as a mixture, and by lecithin in combination with bile salts. In the range of concentrations found in bile, bile salts alone solubilized only about one third as much cholesterol as might be found in comparable concentrated human gallbladder bile. Between 0.023 and 0.046 mmoles of cholesterol were solubilized per mmole of bile salts. Addition of lecithin to a bile salt solution increased the quantity of cholesterol dissolved to values observed in bile; 0.36 mmoles of cholesterol was solubilized per mmole of added lecithin. Cholesterol solubilization by lecithin was independent of the concentration and nature of the bile salt.

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Inhibition of Heat-Induced Denaturation of Serum Proteins by Mixtures of Nonsteroidal Anti-Inflammatory Agents and Amino Acids (32984)

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Recent studies by Nielsen *et al.*(1) have shown that 1-day-old chicks given diets deficient in zinc developed bone and joint deformities similar to those found in patients suffering from rheumatoid arthritis (RA). The addition of zinc, histidine, or histamine reversed the condition(1), as did antiarthritic agents such as aspirin, cortisone, indomethacin, and phenylbutazone.[1] Recent findings by Gerber and Gerber(2) show that patients suffering from RA have lower than normal blood serum concentrations of histidine (1.90 ± 0.03 mg/100 ml, n= 215 vs 1.37 ± 0.04 mg/100, ml n = 80). Feeding a high protein diet exerts a protective action against adjuvant-induced arthritis in rats(3). Moreover, it is known that certain tryptophan metabolites are found in excess in the urine of patients with RA(4). The excess, however, is not specific for RA and is seen in other conditions including bladder cancer, porphyria, scleroderma, systemic lupus erythematosus, and pregnancy(5). These findings indi-

[1] Chem. Eng. News **45**, 14 (1967).

TABLE I. Inhibition of Protein Denaturation.

Compound	Inhibition (%)		
	5×10^{-4} *M*	5×10^{-5} *M*	5×10^{-6} *M*
Indomethacin	93.0 (4)[a]	18.4 (2)	0 (2)
Phenylbutazone	93.1 (4)	11.0 (2)	2.6 (2)
Flufenamic acid	99.7 (4)	41.6 (2)	3.7 (2)
l-Serine	1.3 (2)	3.0 (2)	1.2 (2)
l-Phenylalanine	1.2 (2)	0.4 (2)	0 (2)
l-Tryptophan	5.1 (2)	3.2 (2)	0 (2)
l-Tyrosine	1.3 (2)	5.5 (2)	2.0 (2)
l-Histidine monohydrochloride	3.9 (2)	0	0
l-Cysteine	5.2 (2)	1.6 (2)	5.3 (2)
Histamine diphosphate	0 (2)	0 (2)	1.0 (2)

[a] No. of replicates given in parentheses.

cate that in RA there may be some difficulty in the metabolism of zinc, histidine or histamine, and suggest that histidine or histamine may reduce denaturation of serum proteins in rheumatoid patients (and therefore reduce the production of rheumatoid factor), thus alleviating certain arthritic syndromes.

It, therefore, seemed relevant to present results found in studies in which we defined the effect several amino acids have on the *in vitro* heat-induced denaturation of bovine serum protein (BSA); a phenomenon that Mizushima(6) observed to be readily reversed by various anti-inflammatory agents.

Methods. Solutions containing mixtures of drugs and amino acids at 10^{-3} *M* concentrations were prepared by dissolving the appropriate drug and amino acid in H_2O (using 0.01 *N* NaOH to dissolve insoluble compounds) and adjusting the pH to 5.3. These solutions were then diluted 1–10 and 1–100, respectively, for 10^{-4} *M* and 10^{-5} *M* concentrations.

Two ml of BSA (1.0% crystalline bovine serum albumin dissolved in 6.67 *M* sodium phosphate buffered isotonic saline, pH 5.30) and two ml of drug solution (10^{-3}, 10^{-4}, 10^{-5} M prepared by dissolving in water or, if insoluble, by dissolving with 0.1 *N* HCl or 0.1 *N* NaOH and readjusting to pH 5.3) are mixed and incubated for 20 min at 25°C in a water bath. The mixture is then heated for *exactly* 4.0 min at 67°C and reimmersed in the 25°C bath. After 15 min the degree of turbidity of the mixture is determined by reading absorbance at 660 mμ in a Beckman model B spectrophotometer using 1:1 diluted buffer (6.67 *M* sodium phosphate buffered saline pH 5.30) as a blank.

Percentage inhibition of denaturation is calculated by the following expression:

$$100 - \left[\frac{\text{Absorbance (with drug) at 660 m}\mu}{\text{Absorbance (BSA alone) at 660 m}\mu} \times 100\right] = \% \text{ inhibition}$$

Results. Data in Table I show that, at concentrations of 5×10^{-4} *M*, the anti-inflammatory agents, indomethacin, phenylbutazone, or flufenamic acid readily inhibit denaturation of BSA induced by heat. Lower concentrations are less effective, in general, although flufenamic acid shows approximately 40% inhibition at 5×10^{-5} *M* concentration. At 5×10^{-6} *M* concentrations these agents are inactive. Histamine and the amino acids listed in Table I were ineffective in preventing denaturation of BSA.

Although there is a slight increase in inhibition with 5×10^{-5} *M* mixtures of phenylbutazone and histidine or phenylbutazone and histamine, a comparison of the results in Table II with those in Table I shows that mixtures of other anti-inflammatory agents and amino acids were no more effective in inhibiting the denaturation of BSA at 5×10^{-4}, 5×10^{-5}, or 5×10^{-6} *M* than anti-inflammatory drugs alone. From Table II it appears that 5×10^{-5} *M* concen-

TABLE II. Inhibition of Protein Denaturation.

Mixture (drug + amino acid)	Inhibition (%)		
	5×10^{-4} M[a]	5×10^{-5} M	5×10^{-6} M
Indomethacin + *l*-serine	85.9[b]	2.7	0
Indomethacin + *l*-histidine monohydrochloride	87.9	16.9	8.1
Indomethacin + *l*-phenylalanine	99.5	27.7	0
Indomethacin + *l*-cysteine	78.5	0	0
Indomethacin + *l*-tryptophan	87.3	5.5	0
Indomethacin + histamine diphosphate	85.1	18.7	7.6
Indomethacin + *l*-tyrosine	99.6	20.7	0.2
Phenylbutazone + *l*-serine	86.5	12.1	2.0
Phenylbutazone + *l*-histidine monohydrochloride	98.9	25.4	1.4
Phenylbutazone + *l*-phenylalanine	91.6	12.4	0
Phenylbutazone + *l*-cysteine	99.3	16.2	4.0
Phenylbutazone + *l*-tryptophan	86.9	12.1	3.0
Phenylbutazone + histamine diphosphate	99.6	25.9	0
Phenylbutazone + *l*-tyrosine	94.2	9.2	0.7
Flufenamic acid + *l*-serine	99.6	41.8	5.5
Flufenamic acid + histidine monohydrochloride	99.7	42.8	1.0
Flufenamic acid + *l*-phenylalanine	99.6	43.3	2.0
Flufenamic acid + *l*-cysteine	100.0	27.3	2.3
Flufenamic acid + *l*-tryptophan	99.6	42.4	5.7
Flufenamic acid + histamine diphosphate	99.7	48.2	6.0
Flufenamic acid + *l*-tyrosine	99.6	41.4	1.3

[a] Final concentration of each agent.
[b] All determinations in duplicate.

trations of l-cysteine decrease the ability of indomethacin and flufenamic acid to inhibit denaturation of BSA. Although the results with flufenamic acid and cysteine, in particular, are quite apparent, the significance of these values is questionable since the amino acids do not potentiate the action of antiarthritic drugs at 5×10^{-6} *M* nor does cysteine affect the activity of flufenamic acid at 5×10^{-4} *M*.

Discussion. Metabolic difficulties in handling histamine, histidine, and perhaps other amino acids have led some investigators to propose that these agents may have an alleviating effect on certain syndromes found in patients suffering from RA. This effect could be a result of the ability of histidine or histamine to prevent the denaturation of serum protein, which may be related to the production of rheumatoid factor. The anti-inflammatory agents, indomethacin, phenylbutazone, and flufenamic acid inhibit heat-induced denaturation of BSA *in vitro*; however, histamine, histidine, and several other amino acids were ineffective. Mixtures of drugs and these amino acids were no more effective than drugs alone. Whether supplying sufficient quantities *in vivo* of any of these amino acids or zinc might have a beneficial effect on the RA syndrome is a matter for further investigation since data in this report demonstrate that amino acids are ineffective in ameliorating an *in vitro* model of inflammation.

Summary. Phenylbutazone, indomethacin, and flufenamic acid inhibit, *in vitro*, the heat-induced denaturation of bovine serum albumin at concentrations of 5×10^{-4} *M*. Histamine diphosphate and the amino acids, *l*-serine, *l*-phenylalanine, *l*-tryptophan, *l*-tyrosine, *l*-histidine monohydrochloride and *l*-cysteine were neither effective in inhibiting protein denaturation nor in potentiating the effects of the nonsteroidal anti-inflammatory drugs when mixtures of drugs and amino acids were tested together.

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Hemorrhagic Diathesis in Laboratory Rodents* (32985)

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A disease characterized by spontaneous hemorrhage and death affected a large portion of newly established breeding colonies of specific-pathogen-free (SPF) mice and rats. The subsequent occurrence of an identical problem in similar mice and rats at other laboratories and the increasing use of the SPF animal for research emphasized the importance of this problem and the need to carefully define experimental animals.

Clinical history. Our Division maintains approximately 2,000 pairs of breeding mice and rats and a total of 30,000 rodents in 40 animal rooms. All are housed as pairs in plastic cages fitted with stainless steel tops; white pine shavings are used as bedding; a commercial diet with a 10% fat content is fed; water and bottles are sterilized by autoclaving.

The hemorrhagic disease occurred only in two rooms that contained cesarean-derived mice and rats.

The affected rat colony (SD/Anl-SPF) was started from 54 male and 54 female germfree Sprague-Dawley weanling rats purchased from a commercial breeder. They were removed from plastic isolators prior to shipment to the Laboratory and at 12 weeks of age they were paired and bred. Each pair produced a litter with very few losses among the progeny, and none among the adults.

At the same time an SPF mouse colony (CF no.1/Anl-SPF) was initiated by foster nursing cesarean-derived CF no.1/Anl neonates on purchased germfree dams. Twenty-six females and 13 males were removed from plastic isolators and bred at 8 weeks of age.

At 16 weeks of age, one of the original SPF male rats bled to death from a minor facial laceration. Within the next 4 weeks, 43 more males and three females died. Most showed no signs of illness before death. A few males showed anemia and hemorrhage of one or more of the following: conjuctiva, external nares, urethra, facial skin, subcutis tunica vaginalis visible through the scrotum. A few also had posterior paralysis or paresis. Bleeding from the vulva was noted in the affected females.

During the 7 months after the first death, 120 adult rats died; 98 males and 22 females. The signs remained constant but their severity diminished with time. Approximately two-thirds of the deaths occurred within the first 90 days.

When the SPF mice were 10 weeks old, four of the males died. Wthin 10 days, the remaining nine males died with signs that included dyspnea, depression, and generalized anemia. A few days after the last male died, a female died showing similar signs preceding death.

During the same 7 months that the problem was studied in the rat colony, 200 mice died; 148 males and 52 females.

Materials and Methods. Pathology. All

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