rates. This mechanism of bacterial elimination would therefore be highly efficient, requiring only small amounts of S-IgA. This may account for the low titers of S-IgA and other immunoglobulins that are usually detected in secretions.

There are data which imply that S-IgA functions in this manner in vivo. Bacteria present in human saliva are known to be coated with S-IgA (*9*), and thus they would be expected to be in a state of suppressed adherence. This appears so, for epithelial cells scraped directly from human cheeks contain only 10 to 15 bacteria per epithelial cell even though they are continuously exposed to concentrations of *S. salivarius, S. sanguis, S. mitis*, and other indigenous species approaching 10^8 cells per milliliter of saliva (*10*). However, as observed in this and a previous study (*3*), if epithelial cells are washed and incubated with species of these streptococci, grown in vitro, hundreds of bacterial cells attach to an epithelial cell within minutes. However, the high adherence in vitro of *S. salivarius* strain 9GS2 was inhibited by fourfold concentrated unfractionated parotid saliva (Table 1). The observed inhibition of adherence of this strain by parotid saliva appears to be mainly due to S-IgA, for addition of monospecific antiserum to IgA (*11*) reduced its inhibitory action (Table 1). Unfractionated saliva did not affect the adherence of serotypically different *S. salivarius* strain SSD3, which it did not agglutinate (Table 1). Consequently, it appears that parotid saliva constituents other than S-IgA have limited or no effect on the adherence of *S. salivarius*.

The studies of Freter also substantiate the concept of S-IgA–mediated inhibition of bacterial adherence (*12*). He observed that induced coproantibodies (primarily S-IgA) were protective against infection by *Vibrio cholerae*. When immune animals were challenged with *V. cholerae*, the organisms tended to remain free in the lumen, whereas in nonimmune animals they were adsorbed to the mucosa. Freter also observed that immune serum inhibited the attachment of *V. cholerae* to intestinal mucosa in vitro (*13*), but he did not consider the observed inhibition of bacterial adherence to be related to the protective action of intestinal S-IgA.

The mechanism proposed for the protective action of S-IgA can explain how this immunoglobulin functions in the disposal of bacterial antigens without requiring the bactericidal or opsonizing properties of other immunoglobulins. Its potential to influence the ecology of indigenous bacteria as well as of pathogens can explain the emergence and disappearance, which occur on naso-oral mucous surfaces and in the intestinal canal, of specific serotypes or phage types of bacteria (*14*). Moreover, it offers a rational basis for understanding how relatively small numbers of exogenous pathogens introduced as droplets on a mucous membrane can gain numerical predominance over the existing indigenous microflora. If one considers the indigenous flora to be in a state of partially suppressed adherence mediated by S-IgA, exogenously introduced serotypically distinct pathogens would be expected to enjoy a temporary immunologic selective advantage enabling them to adhere and colonize unimpeded by S-IgA. Once they attained a mass sufficient to stimulate production of specific S-IgA, their colonization would be inhibited, and an immune state created. However, should their colonization progress slowly so as to provide only a weak antigenic challenge, the small amounts of S-IgA produced would affect the pathogen comparably to indigenous bacteria, and a transient balanced or "carrier" state would result.

The secretions bathing mucous surfaces have been recognized to have a cleansing function. The concepts advanced in our report imply that immunoglobulins in these secretions facilitate the clearance of bacteria, and, as such, provide the basis of a primary defense mechanism. Should organisms elude this defense, and subsequently invade the host tissues, systemic defense mechanisms involving serum antibodies, complement, phagocytes, and other recognized systemic factors of immunity would assume importance.

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Nitrogen Excretion in Cockroaches: Uric Acid Is Not a Major Product

Abstract. *Thin-layer chromatographic and enzymatic spectrophotometric analyses have failed to detect uric acid in fecal extracts from American cockroaches on specified diets. Uric acid appears in the excreta when it is a dietary constituent, although most of it is apparently absorbed and stored internally. Surprisingly, ammonia is a major excretory product.*

Uric acid is generally thought to be the major nitrogenous waste product excreted by cockroaches (*1*), and for years students have been taught this (*2*). In contrast, our analyses with thin-layer chromatography (*3*) and enzymatic spectrophotometry (*4*), have shown that little or no uric acid is present in the nitrogenous materials excreted by the American cockroach, *Periplaneta americana* (L.). We have confirmed the presence of small quantities of materials, activated by ultraviolet light, in cockroach excreta (*5*) and have shown that they are not uric acid, nor are they allantoin, allantoic acid, urea, or any of the common purines, pteridines, or pyrimidines.

Table 1. Comparison of uric acid consumption and excretion in male and female American cockroaches maintained on diets containing [2-^{14}C]uric acid. The values are expressed as averages ± standard errors; E/C (%) is (counts per minute excreted divided by counts per minute consumed) × 100; UAE/C (%) is (counts per minute of uric acid excreted divided by counts per minute consumed) × 100.

Diet*	Consumption	Excretion			
	Total uric acid activity consumed (count/min per roach per week)	Total activity excreted (count/min per roach per week)	E/C (%)	Total uric acid activity excreted (count/min per roach per week)	UAE/C (%)
		Males			
42% protein + 1% uric acid	1,062,258 ± 40,385	39,320 ± 1,116	3.7	16,571 ± 535	1.6
42% protein + 4% uric acid	2,075,878 ± 136,123	92,422 ± 3,268	4.5	49,088 ± 5,914	2.4
		Females			
42% protein + 1% uric acid	955,298 ± 161,541	29,788 ± 7,364	3.1	14,348 ± 3,374	1.5
42% protein + 4% uric acid	2,979,585 ± 158,810	113,723 ± 22,582	3.8	58,334 ± 11,352	2.0

* The 42 percent protein diet is equivalent to the 50 percent diet of Haydak (*10*) diluted to 42 percent with Whatman cellulose powder. [2-^{14}C]Uric acid was incorporated into the diets. The amount of diet consumed and excreta produced were determined. Excreta (and fat body) were lyophilized, extracted with 0.6 percent $LiCO_3$, separated on 1-mm MN300 cellulose thin-layer chromatography plates (*3*), with the spot corresponding to uric acid being removed and placed in Bray's aqueous scintillation counting solution (*11*) for counting.

Since uric acid could not normally be detected in the excreta even when individuals were maintained on 91 percent casein protein diets, the fate of dietary uric acid was followed. Diets containing more than 1 percent uric acid resulted in the detection of small amounts of uric acid in the feces, but the amount excreted was consistently lower than that consumed. For more precision, [2-^{14}C]uric acid was fed to cockroaches, and their excreta and fat body were collected for assay in a scintillation counter. In males and females maintained on a diet of 42 percent casein protein plus 1.0 percent uric acid (Table 1) only 3.1 to 3.7 percent of the radioactivity consumed was excreted, and only about 1.5 percent of it was attributable to uric acid. Somewhat higher levels of total radioactivity and uric acid were excreted when dietary uric acid reached 4.0 percent. Contrarily, the fat body became quite radioactive, 40 percent of which could be attributed to uric acid. This suggests that dietary uric acid was extensively absorbed and stored in the fat body. These findings agree with those obtained when [^{14}C]adenine was injected into the cockroach *Leucophaea maderae* in which it was rapidly metabolized and preferentially stored in the fat body as ^{14}C-labeled urates with little radioactivity being excreted (*6*). Injection of [^{14}C]uric acid into *P. americana* also gave presumptive evidence of urate storage, but some $^{14}CO_2$ was produced (*7*).

In view of these results, it appears that previous reports of uric acid excretion in *P. americana* may be attributed to cannibalism prior to fecal collection. Cannibalism is a common occurrence in this species (*8*) and could provide a source of dietary uric acid. Earlier reports (*1*) have not indicated that it was considered. Other possible contributing factors are contamination of feces with blood or lost body parts and excreta analysis with methods that are not specific enough to be conclusive. For example, the silver staining methods used by some workers (*1*) to detect uric acid give a positive response with two of the ultraviolet-activated fecal materials mentioned above.

This investigation also showed that ammonia is a major nitrogenous compound eliminated by this insect (Table 2). Under various dietary regimes ammonia accounted for 12 to 91 percent of the total nitrogen excreted by males and 12 to 75 percent of that excreted by females. The data suggest that, at least with those insects on high protein diets, amino groups may be the source of the ammonia nitrogen.

Large-scale release of ammonia by an insect that is a very popular physiological model raises the question of why it has not been observed previously. Perhaps the main reason is that dry or lyophilized excreta have been examined, whereas it is only wet excreta that contain sizable amounts of ammonia. A subsidiary reason may be that it has not been sought because the so-called Needham's rule (*9*) has led researchers not to expect significant ammonia release by a terrestrial insect.

Table 2. Comparison of total, dry fecal, and ammonia nitrogen eliminated from male and female American cockroaches maintained on various diets; NH_3/total (%) is (ammonia nitrogen divided by total nitrogen) × 100.

Diet	Total nitrogen excreted (micrograms per roach per week)	Fecal nitrogen* excreted (micrograms per roach per week)	NH_3 nitrogen† volatilized (micrograms per roach per week)	NH_3/total (%)
		Males		
Dog food	1188	1019	169	14
Cellulose + 5% protein‡	1171	100	1071	91
Dextrin	246	194	52	21
Dextrin + 5% protein	534	329	205	38
24% protein§	700	400	300	43
50% protein	963	650	313	32
79% protein	1819	756	1062	58
91% protein	2013	525	1487	74
		Females		
Dog food	1969	1731	238	12
Cellulose + 5% protein‡	952	241	711	75
Dextrin	446	288	158	35
Dextrin + 5% protein	582	470	112	19
24% protein§	681	563	118	18
50% protein	1150	969	181	16
79% protein	1588	738	850	54
91% protein	2289	783	1506	66

* Determined by a micro-Kjeldahl method (*12*) on lyophilized samples of fecal material pulverized in a dental amalgamator. † Determined from air passed over 40 cockroaches in a closed container. The air was collected and scrubbed continuously in a glass-bead column containing 40 ml of 2*N* HCl. Micro-Kjeldahl (*12*) and direct nesslerization (*13*) were used to quantitate ammonia. ‡ Whatman cellulose powder + 10 percent yeast extract hydrolysate. § Casein protein diets, according to Haydak (*10*).

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Cholesterol Solubility in Lecithin–Bile Salt Systems

Abstract. *The method of sample preparation can markedly influence the rate of dissolution and attainment of supersaturated states of cholesterol. The equilibrium solubility of cholesterol, studied as a function of its physical state in a model bile system, is almost half that of previously accepted values. Slow attainment of the equilibrium state may have acted to bias previous studies. Extrapolation of our data to the clinical situation reveals that many persons considered normal by present standards actually possess bile that is supersaturated with respect to cholesterol and are thus potential gallstone formers.*

The solubility of cholesterol in bile salts (*1*), in phospholipids (*2*), in mixtures of both (*1, 3, 4*), and in human or animal biles (*4, 5*) has been the subject of many investigations. The data presented in these studies show a variation in the equilibrium solubility of cholesterol. Since choiesterol has been reported to exist in different crystalline forms, which vary in crystal lattice parameters and extent of solvation (*6*), it is conceivable that the differences in equilibrium solubility can be attributed to differences in the solid state properties of cholesterol.

We undertook studies to monitor the solubility of cholesterol as a function of its physical state in a lecithin–bile salt system. The bile salts (conjugated bovine) and lecithin (egg) were purified (*7*) and used in a constant molar ratio (3.5/1), designed to mimic their biliary ratio. Several different forms of [4-^{14}C]cholesterol were produced by crystallization from aqueous and nonaqueous solvent systems, vacuum drying, or simultaneous precipitation (coprecipitation) with lecithin and bile salt. X-ray diffractometry, infrared spectroscopy, microscopy, and thermal gravimetric and differential thermal analytic methods were used to characterize these materials. An excess (20 mg ml^{-1}) of the crystalline cholesterols was added to a sterile lecithin–bile salt micellar system (37°C, *p*H 7.4, 0.05*M* phosphate buffer). The cholesterol solubility in the coprecipitate system was followed after addition of the appropriate concentration of buffer. Solubility was monitored by liquid scintillation counting of a filtrate obtained by passage through a membrane system (Millipore HA and AP20).

The results in Figure 1 show that changing the physical state of cholesterol can markedly influence its dissolution characteristics. The coprecipitated cholesterol, existing in an amorphous state dispersed with the lecithin and bile salt molecules, dissolves readily due to the instantaneous formation of lecithin–bile salt–cholesterol micelles after addition of aqueous solvent. The rapidity of this process may be ascribed to the ease with which the amorphous state may be disrupted by the solvent, as well as to the fact that the rate of transport of the cholesterol to and into the micelle is effectively infinite because the micelle is formed around it. The anhydrous cholesterol dissolves at a much greater rate and obtains a higher metastable solubility level than does the hydrate. These higher levels can be reduced by the nucleation of the truly stable, lower-energy hydrous species, with subsequent relief of the supersaturation by precipitation. The rate of conversion is influenced by the presence of crystal growth poisons and the extent of supersaturation. Nucleation and growth of the hydrate from the anhydrous species become apparent after 5 days, while equilibrium solubility in this system is approached in approximately 12 days. It is easy to see how one could be misled into thinking equilibrium had been obtained if the solubility were determined solely at several hourly intervals. Thus, it is of importance to adequately define the physical as well as the chemical nature of the substance under study, before and after the solubility determination, so that the thermodynamic parameters can be assigned to the proper species. Knowledge of the physical state allows for meaningful expression of equilibrium solubility.

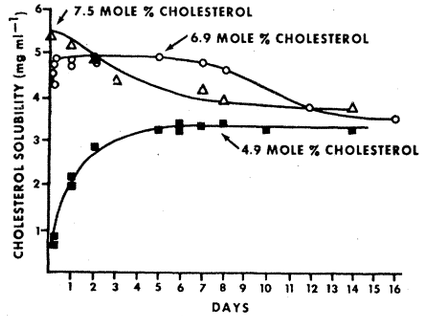

Fig. 1. Dissolution behavior of several forms of cholesterol in lecithin–bile salt system; coprecipitated (triangles), anhydrous (circles), and monohydrated (squares) cholesterol. The inserted mole percentages express the maximal solubility obtained with each form of cholesterol.

The equilibrium solubility of cholesterol in these model systems, designed to mimic the biliary lipid milieu, is in the order of 5 mole percent [unit defined in (*7*)]. This may be of medical significance, because the cholesterol concentrations in the bile of normal humans (no gallstones) have been reported to be far greater than 5 mole percent (*7, 8*). This would imply the presence of supersaturation in many normal biles and raise questions about the factors responsible for the maintenance of the supersaturation. The potential for cholelithiasis may be far greater than is presently imagined. The "forgotten," water-soluble components of bile, which contribute little to the solubilization of cholesterol, may be the determinants of whether or not cholesterol precipitates from supersaturated media.

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