TABLE 1

LIVER CHANGES INDUCED BY AAF; INFLUENCE OF URACIL AND THIOURACIL

	No. animals	Duration (days)	Liver changes (hepatomas and cholangiomas)	Mean liver wt* (g/100 g body wt)
Group I AAF	9	310–409	9	6.6 ± 0.71
Group II AAF and thiouracil	16	385–415	1	3.7 ± 0.23
Group III AAF, thiouracil, and uracil	5	407	3	6.5 ± 1.98
Group IV AAF, and uracil	5	385–409	5	8.2 ± 1.41

* Standard error = $\sqrt{\frac{\Sigma(\bar{x})^2}{n(n-1)}}$.

tion of uracil appeared to overcome this protection, 3 of 5 animals so treated showing marked liver changes (Group III). The protective action of thiouracil is reflected also in the liver weights, which indicate roughly the extent of liver changes induced by AAF. The mean liver weight of animals receiving uracil and thiouracil simultaneously (Group III) was the same as that of the animals treated with the carcinogen alone (Group I). Those given uracil and AAF (Group IV) exhibited the highest liver weights, suggesting that uracil intensifies the effect of the carcinogen on the liver.

The thyroid hyperplasia induced by thiouracil was not inhibited by simultaneous administration of uracil (Table 2). The thyroid weight and the histologic picture did not differ in the two groups.

TABLE 2

THYROID WEIGHT OF RATS TREATED FOR 35 DAYS WITH THIOURACIL, AND WITH THIOURACIL PLUS URACIL, RESPECTIVELY

	No. animals	Mean thyroid wt (mg)	Mean thyroid wt (mg/100 g body wt)
Group I Thiouracil	9	32.7	14.2
Group II Thiouracil and uracil	11	30.3	14.4

In a previous communication (*1*) the question was discussed as to whether the effect of thiouracil in counteracting the hepatic carcinogenic effect of AAF might be dependent upon the induced hypothyroidism. The present observation that uracil, in the dosage employed, does not prevent the thyroid hyperplasia induced by thiouracil, whereas it almost completely overcomes thiouracil protection against the hepatic carcinogenic action of AAF, lends additional support to the view that this "anticarcinogenic" action of thiouracil is not due to the induced hypothyroidism.

The findings suggest the possibility that uracil may be utilized by, and be a nutritional requirement for, liver cells exposed to the carcinogenic action of AAF, and that thiouracil may act as an antimetabolite under these circumstances, as it does in *Tetrahymena geleii* (*2*). Further experiments based on this working hypothesis are in progress.

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Homologous Mechanism of Bactericidal Action and Gram-Staining

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As has recently been shown (*1*), a positive correlation exists between the affinity to wool of water-soluble substances and their antibacterial effect. Further experiments (*2*) have specified this correlation as follows: the higher the affinity to wool of a water-soluble substance at pH 7, the higher is its bactericidal action. This correlation can be demonstrated for chemically different compounds within the anion-active and the cation-active series, respectively. Finally, comparative study of 18 chemically different compounds (*3*) has led to the conclusion that the affinity to wool of a water-soluble substance is a measure of the bactericidal action against gram-positive bacteria. Some examples are given in Table 1.

Thus it is possible to predict the bactericidal effect against gram-positive bacteria of water-soluble, thermostable compounds by the determination of their affinity to wool. This determination (*3*) is carried through by treating one g of wool with 50 ml of a neutral aqueous solution of 0.2 g of the compound in question at 90° C for 10 min and weighing the wool sample before and after treatment to 10^{-4} g.

If wool is degraded with 0.15 *N* Na_2CO_3 at 80° C, first there is a diminution of the basic groups, followed by a "neutralization" of the wool proteins, and finally there is a prevalence of the acid groups. In accordance with these steps of degradation, anion-active (acid) compounds show a decrease in their affinity to wool, whereas with cation-active (basic) compounds an increase in their affinity is found.

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TABLE 1

Substances	Affinity to 1 g wool (mM)	Relative affinities to wool	Minimum (gram +) bactericidal conc *Staph. aureus haemolyticus*	Relative bactericidal activities
(*a*) Anion-active				
$CuCl_2$	0.0175	1	1: 25–1: 50	1
"Eulan new" (IG) Na-salt of tetrachlor-dihydroxy-triphenylmethane-sulfonic acid	.0500	2.8	1: 240	6.4
"Mitin FF" (Geigy) Na-salt of dichlorphenyl-(chlor/chlorsulfophenoxy/phenyl) urea; pure active ingredient	.0908	5.8	1: 200–1: 400	8.0
$HgCl_2$*	.280	16.0	1: 400–1: 800	16.0
(*b*) Cation-active				
"Sapamin KW conc" (Ciba) analog of $C_{17}H_{33}—CO \cdot NH \cdot CH_2CH_2 \cdot N(C_2H_5)_2$	.0282	1	1: 800	1
"Desogen"* (Geigy) dodecyl-methyl-phenyl-trimethyl-ammonium-methosulfate	0.0741	2.6	1: 6400–1: 12800	12

* These substances are also bactericidal against gram-negative bacteria. This fact shows that, after a high enough bactericidal activity against gram-positive forms, and also after a high enough affinity to wool value of the same substance, a correlation exists with the bactericidal activity against gram-negative bacteria also.

In this way, wool proteins[2] can be prepared which in their average IP correspond to gram-negative (pH 5) and to gram-positive (pH 2.8) bacteria, respectively (*4*). Comparing the affinity value of a compound to untreated wool (IP, pH 5) and then, for instance, to a 2-hr degraded wool-protein (pH, approx 2.8), it can be stated: The greater the first value in comparison to the second, the greater is the bactericidal effect of this compound against gram-negative bacteria as compared to its effect against gram-positive bacteria, and vice versa. This is shown in Table 2 for examples of the anion-active and cation-active series.

This indicates that the different bactericidal effects of a compound against gram-negative or gram-positive bacteria are due to its affinity to the bacterial body. Similarly, the classification of bacteria into the gram-positive or gram-negative class is based upon the different affinities of the dye gentian violet to the bacteria belonging to the two classes. This different affinity is, in its total result, mainly due to the differences in the IP in gram-negative and gram-positive bacteria. Gentian violet as a basic dye (and also applied in the gram method with phenol, for example, and thus in a weak acidic medium) is bound by the strongly acidic gram-positive bacteria (IP pH 2.8). In contrast, it is only weakly bound and afterwards washed out in gram-negative bacteria (pH 5).[3]

[2] In this publication the comparison is made between 45- and 86-min degraded wool. The comparison between untreated and 2-hr (or even 3–4-hr) degraded wool used in the present communication is more reliable in view of the closer approximation of the IPs of untreated and about 2-hr degraded wool to those of gram-negative and gram-positive bacteria, respectively.

[3] The application of mildly oxidizing mordants in gram stains also contributes to shift the IP of gram-positive bacteria toward the acid range and thus enhances the affinity between dye and bacterium. In the case of gram-negative forms the same mordants leave the IP unaltered.

TABLE 2

Substances	Affinity in mM to 1 g wool degraded with Na_2CO_3 (0.15 *N* at 80° C)		Bactericidal activities against	
	0 min	2 hr	*B. paratyphi B.* (gram −)	*Staph. aureus B. haemolyticus* (gram +)
(*a*) Anion-active				
"Mitin FF" pure	0.0908	0.1580	0	1: 400
$HgCl_2$	.2800	.1878	1: 12800	1: 600
(*b*) Cation-active				
"Sapamin"	.0282	.1889	0	1: 800
"Desogen"	0.0741	0.1544	1: 600	1: 9600

There have been different attempts to explain the mechanism of gram-staining (*cf. 4*), and also the hypothesis has been advanced that gram-positiveness or -negativeness is due to differences in the IP in the two bacterial classes. From our results it is possible to predict the bactericidal activity of compounds by determination of their affinity to wool and to degraded wool with corresponding IPs of gram-negative and gram-positive bacteria. This supports the theory that not only the bactericidal action against gram-negative and -positive bacteria, but also the classification of bacteria according to Gram, is mainly based upon differences in the IP of the two bacterial classes.

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