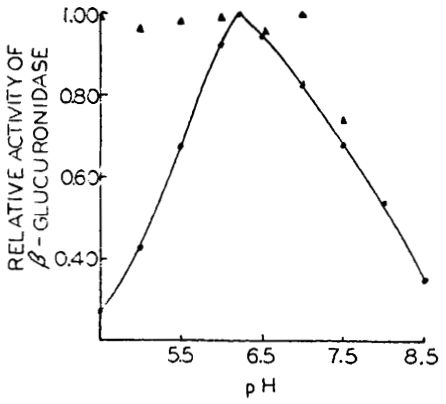

FIG. 4.

Influence of pH on activity of β-glucuronidase produced by *E. coli*.

●—Phenolphthalein-mono-β-glucuronide as substrate.

▲—Estriol-β-glucuronide as substrate.

Some properties of the β-glucuronidase. As the main object of this investigation was to prepare adequate quantities of β-glucuronidase for use in the determination of steroid glucuronides, properties of the enzyme have not been extensively studied. Dialysis of the culture fluid containing the enzyme caused the loss of at least one-half of the activity. This loss of activity was not prevented by controlling the temperature and the pH during dialysis. This bacterial β-glucuronidase is readily inhibited by ammonium chloride, a concentration of 0.1% of this salt causing a 20% reduction of activity. For this reason, the free acid and not the ammonium salt of menthyl-β-glucuronide is used in the culture medium for the preparation of the enzyme. Sodium chloride, in concentrations above 1.0%, decreases the activity of the enzyme appreciably.

Summary. Potent culture fluid preparations of β-glucuronidase are obtained when *E. coli* is grown at 25°C and pH 7.3 in a simple medium containing menthyl-β-glucuronide. The optimum incubation time is 8 days when continuous agitation is employed. The optimum pH for the hydrolysis of phenolphthalein mono-β-glucuronide is 6.2, while that for estriol-β-glucuronide is 4.5-7.0. Bacterial β-glucuronidase is readily inhibited by ammonium ion. Dialysis markedly decreases the enzymatic activity of our preparations. The culture fluid contains phosphatase but no sulfatase activity.

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Biological Activity of Alkali Treated Aureomycin. (18592)

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Recently, the growth promoting effect of aureomycin on the chick(1,2), the turkey(3), the rat(4,5), and the pig(6) have been reported. The general interpretation of these results has stressed the probable effect of the antibiotic on the intestinal flora of the test animal. The inactivity of intramuscularly administered aureomycin or penicillin(1,7) was in accord with this hypothesis.

Data presented by Stokstad and Jukes(2) suggested that aureomycin after microbiological inactivation with alkali was still active for the chick. As these data were not in agreement with the results we had obtained, it was decided to investigate more fully the biological

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TABLE I. Antibacterial Spectrum of Aureomycin, Aureomycin Mash, and Alkali Treated Aureomycin Mash Samples.

	Minimal concentrations* suppressing visible growth after 24 hr incubation				
			Alkali treated mash		
Organism	Aureomycin, γ/ml	Aureomycin mash, γ/ml	pH 13.0, γ/ml	1 cc of 5N NaOH, γ/ml	5 cc of 5N NaOH, γ/ml
Shigella gallinarum	1.56	1.56	1.56	1.56	1.56
Bacillus subtilis	.048	.048	.048	.048	.048
Staphylococcus albus	.195	.195	.195	.195	.195
Staphylococcus aureus	.097	.097	.097	.097	.097
Bacillus cereus	.048	.048	.097	.048	.024
Escherichia coli	1.56	1.56	1.56	1.56	1.56

* The solutions were diluted to equivalent concentrations based on residual antibiotic activity as determined by assay with *Staphylococcus aureus*.

activity of alkali inactivated aureomycin and to study the stability of aureomycin in pure solution and in the presence of certain additives.

Experimental. The paper of Stokstad and Jukes mentioned heating whole aureomycin mash with an excess of 0.5N sodium hydroxide followed by neutralization and concentration. Experiments were carried out as follows: Whole aureomycin mash from an industrial fermentation was divided into four 50 ml portions. Three of the portions were adjusted to pH 13 by the addition of 5N NaOH. One ml and 5 ml of 5N NaOH were added respectively to 2 of the flasks. The volume in each flask was then adjusted to 60 ml. The 3 flasks with alkali were heated with flowing steam in an autoclave for 30 minutes. At the end of this time each sample was extracted with 9 volumes of acid aqueous acetone (1 part 4N HCl, 13 parts acetone and 6 parts water) and the extracts were assayed turbidimetrically for aureomycin activity(8). When compared with the unheated aureomycin mash, the sample which had been adjusted to pH 13 and then heated retained 70% of its activity. The sample receiving 1 ml of 5N NaOH after being adjusted to pH 13 was 52% as active as the control whereas the sample receiving 5 ml of 5N NaOH was 41% as active. From the data it is apparent that even after heating for rather extended periods in alkali, appreciable antibiotic activity remains in the "inactivated" mash. In contrast to this, solutions of pure aureomycin are rapidly inactivated by these procedures.

That the activity remaining is due to unchanged aureomycin is not answered by the above data. To gain further information on this point, the anti-bacterial spectra and the behavior of the alkali treated material on paper chromatography was investigated. The spectra of the extracts of the various alkali treated samples were identical to that of aureomycin (Table I). The diluted mash extracts were applied to 0.5 inch paper strips and developed for 17 hours at room temperature in an atmosphere saturated with N HCl–n butanol (1:1 by volume). The R_f value for the residual antibiotic in all samples was identical with the value of 0.75 obtained by Arnow and Emerick(9) for aureomycin.

The protective effect of the whole mash on aureomycin with respect to inactivation by alkali suggested that perhaps the presence of colloidal materials would exert a similar protective action. In order to test this theory various colloidal substances were suspended in water, a solution of aureomycin hydrochloride was added and the pH of each was adjusted to 9.0 by the addition of 5N sodium hydroxide. The suspensions were placed in the 37° incubator and samples were removed for microbiological assay daily. The results are shown in Table II. It is apparent that the addition of any of a number of colloids or solutions that contain colloidal or suspended

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TABLE II. Protective Action of Various Colloids or Suspended Materials Upon Aureomycin in Aqueous Solution at pH 9.0 and 37°C.

Substance added	Conc., %	Aureomycin conc., %	% destruction at, hr			
			24	48	72	96
0		.1	99.2	99.5	99.8	99.9
India gum	1.0	.1	22	37	41	47
Gum arabic	5.0	.1	3	18	24	36
Gum tragacanth	1.5	.1	27	25	39	41
Egg albumin	5.0	.1	17	22	21	37
Tomato juice	90.0	.025	50	69	78	84

TABLE III. Growth Promoting Activity of Alkali Inactivated Aureomycin on the Chick.

Supplement per kg diet	Avg wt and numbers alive ()		
	Initial, g	15 days, g	28 days, g
1. Basal (1) + 90 γ B_{12}/kg	41(9)	148(9)	347(9)
2. As 1 + 10 mg aureomycin	40(9)	171(9)	369(9)
3. " + 25 mg "	40(9)	171(9)	385(8)*
4. " + 10 mg alkali treated aureomycin	40(9)	147(7)	338(7)
5. " + 25 " " " "	38(9)	145(8)	305(8)
6. " + 50 " " " "	44(9)	150(9)	319(9)
7. " + 100 " " " "	43(9)	168(9)	354(9)

* Significant at 5% level.

materials markedly stabilizes solutions of aureomycin to the destructive effects of alkali.

The results so far presented indicate that treatment of the whole aureomycin mash by alkali under the present conditions does not cause complete inactivation of the antibiotic. The activity reported in the experiments of Stokstad and Jukes could thus be attributed to unchanged aureomycin.

The possibility still existed that the microbiological activity of the mash which was due to residual aureomycin, did not truly represent the activity for the chick and that the alkali treated aureomycin would be active in the latter test. To test this hypothesis the product arising from the treatment of aureomycin with dilute alkali was isolated and tested on the chick. In order to avoid any drastic degradation of the aureomycin molecule, the alkali treatment was carried out essentially as described by Kelsey and Goldman(10). Ten grams of aureomycin hydrochloride was dissolved in 25 ml of water with just enough 10% NaOH to effect complete solution. Two hundred and fifty ml of phosphate buffer (pH 10.5, 2N) was then added. The solution was heated to 90-95° for 5 minutes and then refluxed for 5 minutes (24 hours at 25° effects the same transformation). The mixture was cooled, adjusted to pH 5.4 with hydrochloric acid and the precipitate collected on a filter. The compound was dissolved in dilute alkali, decolorized with 5 grams of Norite A and the solution then adjusted to pH 5.4. The crystalline compound was collected and dried. A small sample was recrystallized from dilute hydrochloric acid. Analyses. C, 50.67; H, 4.90; N, 5.35; Cl, 13.81.

When tested against *Staphylococcus aureus*, the compound exhibited less than 0.04% of the antibiotic activity of aureomycin. The effect of the compound on the growth of the chick was determined as previously described (1). These results are summarized in Table III. The compound is inactive for the chick at the various levels tested.

Discussion. The foregoing data suggest that rather drastic conditions would be necessary to completely inactivate aureomycin in the whole fermentation mash. The residual aureomycin is most probably the active agent in the alkali "inactivated" product of Stokstad and Jukes rather than an antibacterially inert product that retains activity for the

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chick. This view is strengthened by the fact that a compound which is formed from aureomycin by treatment with dilute alkali is inactive for the chick and exhibits only a fraction of the antibacterial activity of aureomycin. These data further support the view that the antibiotic activity of a compound and its growth promoting activity for animals are related. The specific activity of each compound as regards its growth promoting effect is most probably correlated with the antibiotic spectrum of the compound and with the availability and stability of the substance in the intestinal tract of the animal. These factors could account in part for the fact that certain antibiotics are superior to others as growth stimulators.

Several substances when added to solutions of aureomycin stabilize the latter against inactivation by alkali. The exact mode of this action is unknown.

Summary. Alkali treated *Streptomyces aureofaciens* fermentation liquor contained considerable unchanged aureomycin. The pure antibiotic is inactivated by similar treatment. The product arising from mild alkaline inactivation of aureomycin was isolated and found to have essentially no antibacterial activity and no growth stimulating activity for the chick.

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Pathological Changes in Adrenal Cortex of Mouse During Experimental Meningococcal Infection.* (18593)

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Importance has been attached to hemorrhage(1,2) and other changes in the adrenal cortex, particularly the "tubular degeneration" of Rich(3), in patients dying from fulminating meningococcal sepsis. These reports suggested that similar changes might be reproduced in animals experimentally infected with meningococcus.

The following observations are limited to microscopic changes in the adrenal cortex such as changes in the amount and distribution of fat, hyperemia and inflammatory infiltration.

Methods and materials. The meningococcus employed was a type I strain originally isolated from the blood stream of a child acutely ill with epidemic meningitis. A 6-hour culture of the organism was suspended in 4% mucin‡ and mice were inoculated intraperitoneally with 1.0 ml containing from 10^2 to 10^5 organisms. The virulence of this strain had been increased by mouse passage so that < 10 meningococci in mucin produced sepsis which was fatal within 48 hours. Animals which were inoculated with 10^2 organisms and remained untreated were moribund at 24 hours and rarely lived for 48 hours. No

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‡ Granular mucin, type 1701-W, supplied by the Wilson Laboratories, Chicago, Ill. The method of preparation is described by Miller and Bohnhoff(4).

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