mg, however, caused persistent contraversive forced circling and decreased the cholinesterase value of the nucleus caudatus to from 21 to 40% of normal. There was no evidence of significant spread of the DFP within the brain in this group of animals. The administration of atropine sulfate either prevents or stopped these forced circus movements. Persistent forced circling could also be induced with larger doses of from 0.23 to 0.32 mg of DFP and evidence of spread of the DFP was then observed. In this group the injected nucleus caudatus showed ChE activity values of from 18 to 20% of the control whereas remaining parts of the brain revealed symmetrical reduction in ChE activity between 48 to 82% of normal. When still greater amounts of DFP were injected (1 to 10 mg) the spread of this substance progressively increased so that eventually ChE activity was decreased bilaterally and transient circling or death resulted.

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Sensitivity of *Staphylococcus aureus* to Penicillin in Various Media. (22681)

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The composition of the culture medium is of importance in testing the sensitivity of microorganisms to penicillin. The media may contain substances which react with penicillin chemically, thereby reducing its effective concentration. Substances having —SH groups such as thioglycollate, cysteine and glutathione behave in this manner(1,2,3,4). Acetic acid, too, inactivates penicillin by chemical interaction(5). Vitamins such as folic acid and vit. B_6 antagonise the effect of penicillin (6), and ascorbic acid also shows a deleterious effect(7). Glucose may diminish the effect of penicillin when added to cultures of *Staphylococcus aureus*(8,9). Furthermore, the activity of penicillin was decreased by addition of serum to the basal medium(10). On the other hand, certain substances, such as nicotinamide, exhibit synergistic effects with penicillin(8,11).

The present work deals with the effects of the addition of various carbohydrates to culture media, on the sensitivity of *Staph. aureus* to penicillin.

Methods. Test organism and inoculum: *Staphylococcus aureus* Oxford strain, was inoculated into nutrient broth (Difco). After 20 hours incubation at 37°C the cells were harvested and washed 3 times with physiological saline. The washed cells were diluted 1:100 in saline and 0.1 cc added to tubes containing 1 cc of medium. Two different media were employed: (a) Nutrient broth (Difco) and (b) a casein hydrolysate medium of the following composition:

Casein hydrolysate (vit. free)* 10%	3 cc
Carbohydrate	1000 mg
Tryptophan	20 mg
Cystine	10 mg
Thiamine	100 γ
Nicotinic acid	100 γ
Distilled water to make 100 cc	

* Obtained from Nutritional Biochemicals Co. (NBC).

After autoclaving, the following sterile solutions were added in amounts of 0.5 cc each: 1) Salt solution composed of: $MgSO_4 \cdot 7H_2O$ 4%; NaCl 0.125% $FeSO_4 \cdot 7H_2O$ 0.125%, and $MnSO_4 \cdot 4H_2O$ 0.125%. 2) K_2HPO_4 8.7%, and the medium was dispensed aseptically, into sterile tubes (12 x 100 mm).

Penicillin solution: Crystalline penicillin G (Shenley) was used. A concentrated solution containing 10,000 u/cc was kept in the refrigerator for one week; dilutions being prepared freshly for each experiment.

Antibacterial assay. The assays were carried out in small test tubes containing 1 cc of the medium. The penicillin effect was tested by the 2-fold dilution technic using the medium as a diluent for the penicillin. After inoculation with *Staph. aureus* the tubes were incubated at 37°C for various periods of time.

Results. Effect of different carbohydrates *on the sensitivity of Staph. aureus to penicillin*. Nutrient broth and casein hydrolysate media, containing glucose, pyruvic acid, acetic acid or lactic acid were inoculated with *Staph. aureus*. (Carbohydrates were added to the media before autoclaving.) Controls without added carbohydrate were included. Penicillin was added to the tubes in various concentrations, and the degrees of growth inhibition were examined after incubation for 24, 48, 72, and 96 hours. The results are given in Fig. 1, which shows that *Staph. aureus* was most sensitive to penicillin in nutrient broth and in a casein-hydrolysate medium containing pyruvic acid; 0.04 u/cc of penicillin inhibited the growth completely. The degree of inhibition did not change appreciably on further incubation. On the other hand, 0.2 u/cc of penicillin was not inhibitory when a casein hydrolysate medium without added carbohydrate was used. When lactic acid was added to casein hydrolysate, growth appeared in tubes containing 0.2 u/cc only after 72 hours of incubation. The growth of *Staph. aureus* in a medium containing acetic acid was not inhibited by concentrations of 0.15 u/cc after 96 hours. In Fig. 1 the sensitivity of *Staph. aureus* is plotted against incubation time. The straight line obtained in a glucose and penicillin-containing medium suggests the occurrence of a chemical interaction between them. To examine this point further, the following experiment was performed: penicillin in a concentration of 5,000 u/cc was incubated at 37°C in a solution of glucose (50 mg/cc). Penicillin incubated similarly in

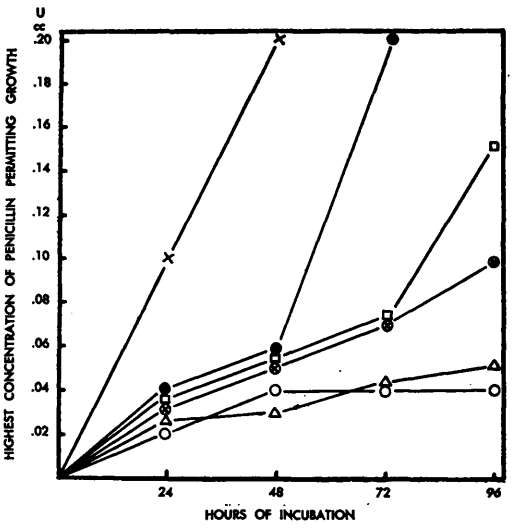
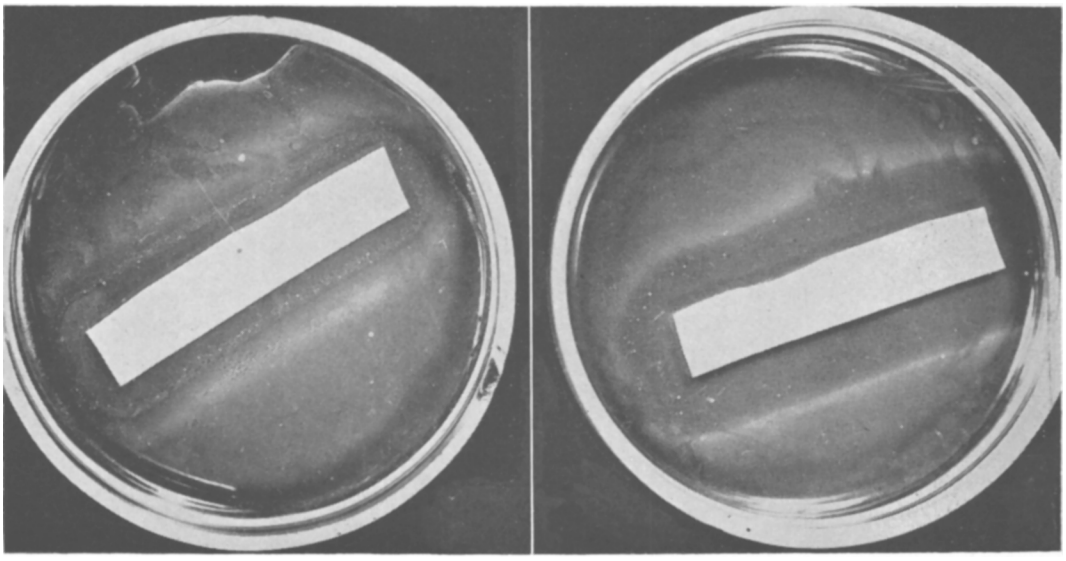


FIG. 1. Effect of carbohydrates on inhibition of *Staphylococcus aureus* by penicillin. Casein hydrolysate medium, —×—; casein hydrolysate medium with lactic acid (1%), —●—; casein hydrolysate with acetic acid (1%), —□—; casein hydrolysate with glucose (1%), —× (in circle)—; casein hydrolysate with pyruvic acid (1%), -△-; nutrient broth (Difco), —○—.



A B

FIG. 2. Effect of glucose on inhibition of *Staphylococcus aureus* by penicillin. (A) Penicillin (5000 u/cc) was incubated with glucose (50 mg/cc) for 5 days at 37°C. Sterile filter paper strips were soaked with the solution and tested for activity against *Staphylococcus aureus*. Photographs taken after growth for 48 hr. (B) Penicillin (5000 u/cc) was incubated with saline solution for 5 days at 37°C. Sterile filter paper strips were soaked with the solution and tested for activity against *Staphylococcus aureus*. Photographs taken after growth for 48 hr.

saline served as a control. After 5 days, pieces of sterile filter paper were soaked in the respective solutions and were placed on agar plates inoculated with *Staph. aureus*. The results are shown in Fig. 2. It is seen that on incubation of penicillin with glucose a marked decrease of the inhibition zone was obtained. No diminution of penicillin activity was found after it was preincubated with lactate; it seems unlikely that a similar chemical reaction between penicillin and lactate had occurred. To eliminate the possibility that the addition of lactic acid stimulated the occurrence of a resistant mutant, the lactate-grown strain was inoculated into nutrient broth and its sensitivity to penicillin re-examined; however, no diminution in sensitivity was observed. Parallel experiments in which the media were sterilized by Seitz filtration were carried out. Table I shows that heating is not the cause of the variation in sensitivity in the different media.

Discussion. Pyruvic acid and glucose when added to media rich in amino acids (supplied as casein hydrolysate), increase the sensitivity of *Staph. aureus* to penicillin. On the other hand, lactic and acetic acids were not effective. The observed interaction between penicillin and glucose explains the reduction in penicillin activity after prolonged incubation in glucose containing medium. No such mechanism is responsible for the low activity of penicillin in lactic acid-medium. Potentiation of the penicillin activity by pyruvate may be explained as follows: (1) The antibiotic interferes with some step in the pyruvic acid metabolism of *Staph. aureus;* in the absence of added pyruvic acid such interference is less pronounced. Or (2) The toxic factor responsible for the antibacterial effect is *not* penicillin *per se* but a compound formed under its influence(12); pyruvic acid contributes to the production of the hypothetic toxic factor. In the absence of pyruvic acid the inhibitor is formed in smaller amounts, hence the lower antibacterial activity. Although the experiments presented do not favor one explanation rather than the other, unpublished work(13) suggests that penicillin interferes with the oxidation of pyruvic acid by cells of *Staph. aureus*. These results indicate that the sensitivity of *Staph. aureus* to

TABLE I. Sensitivity of *Staphylococcus aureus* to Penicillin in Casein Hydrolysate Media Containing Various Carbohydrates.*

	Acetic acid	Lactic acid	Pyruvic acid	Glucose	Nutrient broth	Casein hydrolysate, no carbohydrate (control)
Autoclaved medium	.15†	.2	.025	.1	.025	>.2
Seitz filtered medium	.15	.2	.05	.1	.025	>.2

* Results were read after 96 hr of incubation at 37°C.
† Maximal concentration of penicillin (u/cc) in which growth appeared.

penicillin may be affected by the carbon source of the medium. Similar findings were reported for *E. coli*(14) which was found to be more sensitive to penicillin in the presence of xylose or ribose than with glucose.

Summary. The effect of the composition of the medium on the sensitivity of *Staphylococcus aureus* to penicillin was tested. Pyruvic acid or glucose, when added to a casein hydrolysate medium, increase the antibacterial effect of the antibiotic. Lactic or acetic acids, on the other hand, have no such effect. The possible mechanisms of the enhanced penicillin activity in presence of pyruvic acid are discussed.

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Metabolic Studies of Desaminothyroxine. (22682)

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Desaminothyroxine (3,5 diiodo-4 (3', 5', diiodo-4 hydroxy phenoxy) phenyl-propionic acid) was synthesized by Clayton, *et al.*(1) at the Glaxo Laboratories, Middlesex, England, and by Kharasch *et al.** at the University of Southern California.

Bruice, Winzler and Kharasch(2) reported that in tadpoles (*Rana catesbiana*) at about 24°C, desaminothyroxine possesses 130 times the metamorphosing activity of L-thyroxine. Roth(3) has recently reported that it has 1000 times the activity of L-thyroxine in this test at 16°C. In comparison, Roche(4) has shown desaminothyroxine to have about 75% the antigoitrogenic activity of D-L-thyroxine in rats. Selenkow and Asper(5) reported it to have only 20 to 25% the antigoitrogenic activity of L-thyroxine. No reports on its calorigenic activity in mammals have been found.

* Personal communication.

Method. Measurements of the oxygen consumption of rats were conducted in a 5-unit basal metabolic rate machine at 30°C(6). Individual studies usually lasted 4½ hours, using the first half-hour as a calibration pe-