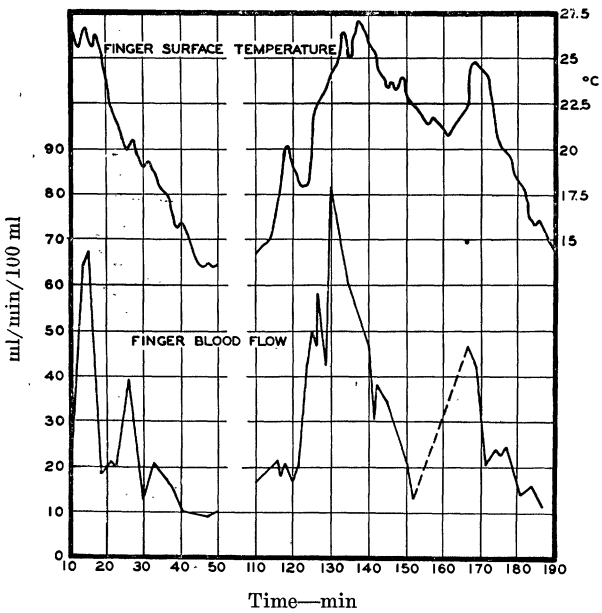

FIG. 3. Blood flow and skin temperature at 7° C ambient. Hand air insulation = 0.30 equivalent clo.

ture is to follow closely the blood flow changes. It is likely that this approximate minimum applies to other areas of the body. This heat loss rate corresponds, for example, to an air insulation over the bare hand of 0.54 equivalent clo[1] (insulation unit) and a temperature difference of 24° C between hand and air; or to a temperature difference of 13° C if the air insulation is 0.30 equivalent clo.[2] Thus, if the temperature of the hand is expected to fall to 21° C the ambient temperature should be −3° C; or 8° C for the higher air movement. If only gross, slow changes of blood flow are to be measured, correspondingly lower heat loss rates may be adequate.

Good blood flow rates may be achieved in normal persons even with high gradients, provided that only the part under investigation is exposed to the cold, and the rest of the body is warm. This can be done by insertion of, say, the extremity of a nude subject into a cold box in a warm room; or by exposure to a cold room of the extremity of a warmly dressed subject. It is true that minute variations of temperature can be measured with high sensitivity apparatus, and that these, with some lag, might represent blood flow more exactly than do the examples given. Such a technique has serious drawbacks, and it would appear advantageous to apply the laws of heat transmission in order to amplify and accelerate temperature changes.

In summary, if direct measurement of blood flow is not feasible, and skin temperature is used instead, the part of the body under investigation must lose heat at a rate of more than about 240 kg-cal/hr/m² for good correspondence between blood flow and skin temperature changes. If local cold stimulus must be avoided, a flow method must be employed.

[1] The clo equation is not strictly applicable to a portion of the body, having been derived for the body as a whole (*1*).

[2] Recent determinations with an electrically heated manikin give an insulation of the air around the hand of 0.54 equivalent clo when that of the whole body is 0.82 clo; and of 0.30 equivalent clo when the average for the body is 0.43 clo.

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The Action of Penicillin on *Bacillus subtilis* Growing in the Absence of Amino Acids[1]

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Gale and his co-workers (*2*) have reported that Gram-positive bacteria are able to assimilate glutamic acid from the medium in which they are grown and to concentrate the free amino acid within the bacterial cell. Gram-negative organisms, on the other hand, appear to be unable to do so. They further observed that when certain strains of *Streptococcus faecalis* and *Staphylococcus aureus* were exposed to penicillin during the logarithmic phase of growth, the ability to concentrate free glutamic acid in the resting cell was lost. Subsequently Bellamy and Klimek (*1*) noted that a strain of *Staphylococcus aureus* which had been trained to grow in extremely high concentrations of penicillin changed from its usual morphology to that of a Gram-negative coccobacillus and acquired the ability to grow in a medium containing no amino acids. Gale (*2, 3*) also found that a strain of *Staphylococcus aureus* trained to grow in a medium deficient in amino acids, acquired *pari passu* a considerable degree of penicillin resistance. He therefore suggested that "penicillin interferes with the mechanism whereby certain amino acids are taken into the cell, and that the sensitivity of the cell to penicillin is then determined by the degree to which its growth processes are dependent upon assimilation of preformed amino acids rather than upon their synthesis."

At least two objections may be raised to this hypothesis. In the first place, some of the Gram-negative coliform organisms are inhibited by high concentrations of penicillin, and yet most of them require no amino acids for growth (*7*). Secondly, *B. subtilis*, a Gram-positive organism with many strains sensitive to penicillin, has been reported in some instances to grow in media containing only ammonia as a source of nitrogen (*5*).

A search was therefore made for strains of *B. subtilis* which would grow in a synthetic medium devoid of amino acids in order to determine whether such strains would be inhibited by penicillin.

[1] This investigation was supported by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

The first few strains investigated grew readily in various synthetic media deficient in amino acids, and under certain conditions were completely inhibited by penicillin. Irregular results were obtained, however, and it eventually became apparent that these could be attributed to the production of penicillinase by the organisms.

A strain of *B. subtilis* (PCI-220-NNRL-B-558) (*6*) was finally obtained which did not produce penicillinase and which grew readily in a synthetic medium, "Base B" (Table 1), containing only ammonium sulfate as a source of nitrogen. In this medium the organism was just as sensitive to penicillin as it was in a "complete" medium (tryptose phosphate broth) irrespective of wide changes in the age of the culture, the size of the inoculum, and the time of reading. (See Table 1.)

TABLE 1

PENICILLIN SENSITIVITY OF *Bacillus subtilis* (PCI-220-NNRL-B-558)

Medium	Inoculum 18-24-hr culture in B.B.		Time of reading in hr	Inhibiting concentration of penicillin u/ml
	Amount and dilution	Approximate no. of viable organisms		
T.P.* broth ..	.2ml 10^{-1}	2×10^4	18	.008
			90	.008
	.2ml 10^{-5}	20	18	.008
	(unwashed)		90	.008
B.B.†	.2ml 10^{-1}	10^3	20	.008
			96	.008
	.2ml 10^{-4}	10	20	.008
	(washed)		96	.008
T.P. agar	.2ml 10^{-1}	2×10^4	18	.01
			90	.05
	.2ml 10^{-4}	6×10^2	18	.01
	(washed)		96	.01
B.B. agar	.2ml 10^{-1}	10^3	20	.01
			96	.01
	.2ml 10^{-4}	10	20	.01
	(washed)		96	.01

* Tryptose phosphate.

† "Base B," composition as follows, wt/liter of medium: KH_2PO_4 – 4.5 g; NaOH – 1.1 g; $FeSO_4 \cdot 7H_2O$ – 0.0139 g; $MgSO_4$ (anhyd.) – 0.02 g; $(NH_4)_2SO_4$ – 0.73 g; glucose – 2.25 g (autoclaved separately); sodium thioglycolate – 0.27 g; thiamine (subsequently found to be unnecessary for growth) – 0.00003 g (autoclaved separately); nicotinic acid (subsequently found to be unnecessary for growth) – 0.001 g; distilled H_2O to 1000 ml. Adjusted to pH 7.4.

Determinations of sensitivity to penicillin were carried out in fluid media as follows: A known quantity of crystalline sodium penicillin G was dissolved directly in the basal medium to give an appropriate concentration. Serial dilutions were then made in 1 ml volumes so that eight tubes, containing concentrations of penicillin varying from 0.15 u/ml to approximately 0.001 u/ml, were obtained. All tubes including a control were inoculated with 0.2 ml of a culture which had been washed three times in sterile saline and resuspended in the basal medium immediately before inoculation. The tubes were incubated at 37° C and were observed at intervals for the appearance of turbidity. Growth in control tubes was usually apparent after 12 hr, and a distinct end point could be read after 18–24 hr.

Agar plates[2] were also prepared containing similar concentrations of penicillin in both the basal medium and tryptose phosphate broth. The plates were streaked with similar washed inocula and incubated at 37° C. The appearance of surface growth was noted, and inhibition was recorded only when no visible colonies occurred.

Precautions were taken to exclude amino acids from the basal medium as completely as possible. Reagent chemicals[3] were used throughout, and test tubes were acid-cleaned and capped with aluminum foil.

In order to determine whether or not this organism elaborated significant amounts of amino acids into the basal medium, a chemical method for the detection of amino acids was employed. By using a modification of the ninhydrin method of Moore and Stein (*4*) it is possible to detect concentrations of glutamic acid smaller than 1 μg/ml added to the basal medium. Excepting proline and hydroxyproline, all of the amino acids as well as ammonia, peptides, and many amines form a colored product with ninhydrin showing an absorption peak at 570 mμ. If the basal medium is aerated for 5 hr at pH 11.5, most of the ammonia is driven off. After neutralization the solution may then be treated with ninhydrin and the color measured with a spectrophotometer at 570 mμ. When this method was employed, the basal medium, thought to be free of amino acids, was found to give a blank value equivalent to that obtained with 0.5–1.0 μg/ml of glutamic acid. It seems likely that most of this color was actually due to residual ammonia, and in any event the value obtained sets an upper limit for possible traces of amino acids in the medium.

In an experiment in which a washed inoculum of this strain of *B. subtilis* was grown in the basal medium for 24 hr, samples of the culture were removed at 6-hr intervals for the following determinations: turbidimetric reading, dry weight of organisms, count of viable organisms, and concentration of ninhydrin-reacting substances in the aerated filtrate. During the first 12 hr, when maximum growth took place, there was no demonstrable increase in ninhydrin-reacting substances in the filtrate, although at 18 and 24 hr, with slowing of growth and death of organisms, small amounts appeared.

After 18-hr incubation there were 200 μg of organisms by dry weight in 1 ml of culture. Since there was no more than 1 μg of amino acid/ml present in the basal medium originally, and since dried bacteria contain an average of 60% protein (*5*), it is apparent that less than 1% of the protein synthesized during growth could be accounted for by amino acids assimilated from the medium.

It is concluded, therefore, that penicillin inhibits the growth of this particular strain of *B. subtilis* by some mechanism other than interference with the assimilation of preformed amino acids by the bacterial cell.

[2] The agar was washed 5 times overnight in distilled water.

[3] Except for Difco sodium thioglycolate.

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A Source of Error in Tracer Experiments with P^{32} [1]

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In dealing with radioactive solutions of high specific activity where total phosphorus is small, the amount of P^{32} adsorbed on laboratory glassware may become significant.

An experiment done in this laboratory to measure this adsorption and to determine the efficacy of silicone-coated glassware illustrates the magnitude of the problem.

A solution of P^{32} containing about 1×10^{-4} mg /ml of total phosphorus was drawn up once into each of four 2-ml pipettes, all new and identical except that two had been coated with silicone. Each of the pipettes was then washed by drawing up 2-ml amounts of distilled water. The wash water from each pipette was collected into two counting sample cups (1 ml each), evaporated, and the number of radioactive disintegrations determined in a Nucleometer.

Average values, in cpm/ml were:

	Coated	Uncoated
Wash 1	390	6,000
Wash 2	110	300
Wash 3	background	110

The count in the first wash of the uncoated pipette is high and variable, depending on both the P^{32} and P^{31} contents, and can cause serious error where it is not suspected.

To avoid this difficulty, we have adopted several techniques. Wherever possible all counting solutions contain a uniform 1 mg/ml of carrier phosphorus at pH 4.5. Where less carrier must be used, care is taken to pipette as rarely as possible and only in solutions containing as much carrier as the procedure permits. When conditions are extremely unfavorable, requiring exceedingly low carrier or high pH values, silicone coating of glassware is necessary.

The coating technique we used was modified from Johannson and Torek (*1*). A 2% solution of "DC 200" was prepared in pure grade CCl_4 (the Dow Company recommends chlorinated organic solvents). The glassware, preferably new, is scrupulously cleaned and heat-dried, completely immersed in the silicone solution, and carefully drained. It is then placed into an oven at 90° C for 2 hr, to evaporate the solvent. The temperature is then raised to 250° C for a period exceeding 4 hr but not longer than overnight. Detailed experiments on the lasting qualities of this type of coating have been done by the Dow Company. This treatment caused no detectable change in calibration.

There is another group of compounds which have been similarly used, called "silanes" (hydrochlorides of unpolymerized silicones). These may be vaporized onto a glass surface, where they react with the film of moisture to form a silicone layer. This process of hydrolysis and polymerization is somewhat difficult to control.

[1] Research carried out at Brookhaven National Laboratory under the auspices of the Atomic Energy Commission.

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Free Amino Acids and Peptides in Frog Embryos

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Relatively large quantities of free amino acids have been detected by two-dimensional paper chromatography in tissues of the mouse (*3, 4*), the human, and the frog (*Rana pipiens*). Each of the normal adult tissues examined appeared to have a distribution of free amino acids characteristic for that tissue. Therefore, it was apparent that the ability of these tissues to maintain the characteristic distributions of these constituents must have arisen at some time during embryonic or postembryonic development.

As the first step in the study of the free amino acids of frog tissues at various stages of development, examinations were made of alcohol extracts of material ranging from ovarian eggs to larvae, corresponding to stages 0, 1, 2, 8, 10, 12, 17, and 21 according to Shumway (*5*). In the last stage examined, there was still no intake of food from the environment. Exactly 260 mg of fresh weight of material was placed in ethyl alcohol, the final concentration of alcohol being 70–73%. The material was homogenized in a ground glass homogenizer, centrifuged, and the supernatant fluid decanted and evaporated to dryness. The residue was thoroughly extracted with 3 ml of distilled water. This was then dialyzed against 10 ml of distilled water in a rocking dialyzer (*2*). One half of the dialyzate was evaporated to dryness and placed on paper for chromatography (*1*), and the other half was chromatographed after hydrolysis with 6 N HCl.

In none of the stages studied were there detectable quantities of free amino acids in the extracts. Several ninhydrin-reactive materials were located at spots which disappeared on acid hydrolysis, and which did not correspond in position to any known amino acids. These were