COMPARATIVE STUDY OF THE BINDING OF SYNNEMATIN AND PENICILLIN BY BACTERIAL CELLS¹

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The specific and irreversible binding of penicillin G with *Staphylococcus aureus* has been shown by several laboratory groups (Cooper and Rowley, 1949; Maass and Johnson, 1949a, b; Rowley *et al.*, 1950). Evidence that the binding reaction is involved in the primary mode of action of penicillin has been reviewed by Cooper (1956).

Although the binding reaction requires an intact penicillin nucleus (Daniel and Johnson, 1954), the penicillin nucleus is not solely responsible for antibiotic activity, since penicillins with different side chains may have widely different bacterial spectra. The present work, a comparative study of the binding of synnematin B and penicillin G, was done to gain information about the role of the side chain in antibiotic activity. The penicillins used were chosen because of their greatly different effectiveness against *S. aureus* (*M. pyogenes* var. *aureus*) (Gottshall *et al.*, 1951).

Binding of the two antibiotics was compared on the bases of amount and rate of binding and stability of the bound complexes, since, if binding is responsible for antibiotic activity, all three of these factors might contribute to the complexing within one generation (Maass and Johnson, 1949b) of enough binding component to cause cell death.

MATERIALS AND METHODS

The procedure used in the growing and harvesting of *Staphylococcus aureus* strain H was basically as described by Schepartz and Johnson (1956). Cells were grown in 4.5 L glass bottles fitted with perforated rubber tubing spargers and charged with 3 L of a medium consisting of peptone, 0.6 per cent; beef extract, 0.3 per cent;

yeast extract, 0.3 per cent; glucose, 0.2 per cent. The bottles were inoculated with about 60 ml of a 24 hr shake flask culture. Yield of Sharples cell paste from 3 L medium was about 11 g after 6 or 7 hr incubation at 30 C.

Nonradioactive potassium penicillin G (1595 International Units per mg) was obtained from Abbott Laboratories. Nonradioactive synnematin (230 units per mg, in terms of the unit of Gottshall *et al.*, 1951) was procured from the Michigan Department of Health Laboratories. Both antibiotics were dissolved in pH 6.3 phosphate buffer (1 per cent w/v) before use. Penicillin concentrations were determined by a *S. aureus* cup-plate assay. Synnematin was assayed by a *Bacillus subtilis* blotter disc method.

To make possible the comparison of activities of the two antibiotics, concentrations were converted from units per ml to μ moles per ml. One μ mole of penicillin G is by definition equal to 594.5 International Units. One μ mole of synnematin is, in the present study, assumed equal to 280 units, as reported by Bhuyan *et al.* (1958).

Penicillium chrysogenum strain 49-133, growing on synthetic medium with added $S^{35}O_4^{2-}$ was used for production of S^{35} -penicillin. The fermentation procedure was that of Schepartz and Johnson (1956). S^{35} -Penicillin was recovered from the medium by a modification of the method of Pan (1954). To 50 ml filtered penicillin broth was added solid ammonium sulfate to 80 per cent saturation. The pH was adjusted to 5.5 with 6 N H_3PO_4 and the broth was extracted twice with 100-ml portions of methyl isobutyl ketone. The ketone extracts were combined and extracted with 25 ml pH 7 (1 per cent w/v) phosphate buffer. Recorder tracings of paper chromatograms (see Brewer and Johnson, 1953, and Schepartz and Johnson, 1956, for details of preparation) showed that about 90 per cent of the radioactivity of the final product was due to penicillin G.

Radioactive synnematin was obtained from

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Figure 1. Growth of *Staphylococcus aureus* in the presence of varying concentrations of synnematin and penicillin. Penicillin, O; synnematin, Δ .

Samples (13 ml) of sterile broth, contained in cotton-plugged 25 by 200 mm test tubes, were given a large inoculum (final cell concentration, 5×10^{-6} g cell dry wt per ml) of *S. aureus* strain H. Immediately, varying amounts of synnematin or penicillin were added to give the concentrations indicated in the figure. (Note that in both large figure and inset 1 μ mole of penicillin equals 106 μ moles of synnematin.) Samples were incubated for 0 to 265 min at 30 C on a rotary shaker. Growth was followed turbidimetrically by reading optical density of the cultures at 615 m μ in a Bausch and Lomb Spectronic 20 spectrophotometer. With this system one optical density unit = 1×10^{-4} g cell dry wt per ml.

Coordinates of the inset (90 min incubation) are the same as in the large graph (265 min incubation). The dotted lines indicate the optical density just after inoculation.

cultures of *Emericellopsis terricola* var. *glabra*, grown on chemically defined medium (Bhuyan and Johnson, 1958) to which $S^{35}O_4^{2-}$ had been added. S^{35} -Synnematin was recovered and purified by means of a charcoal elution column and a Celite 545 partition column. This purification procedure has been described by Bhuyan *et al.* (1958).

All penicillin and synnematin binding was determined by radioactivity assay of cells treated with S^{35} -labeled antibiotics. For count-

ing, radioactive solutions and cell suspensions were diluted five- or ten-fold with alkaline (pH 11) one per cent gelatin. One-milliliter aliquots of the gelatin suspensions were then air dried on copper planchets (5 cm²) and counted with a thin-window or a flowing-gas Geiger-Müller counter. All radioactivity binding by cells was recorded in terms of amount (counts per min, units or μ moles) per g of cell dry weight.

RESULTS

Bacteriostatic level of synnematin and penicillin.

When growing cultures of *S. aureus* were exposed to varying concentrations of synnematin or penicillin, the data presented in figure 1 were obtained. Several differences in the behavior of the two antibiotics toward *S. aureus* are indicated by the figure.

The most obvious difference is, of course, the much greater activity of penicillin. Although the large graph of figure 1 is drawn with the concentration scales selected so that 1 μ mole of penicillin corresponds, on the abscissa, to 106 μ moles of synnematin, the curves of optical density *vs.* concentration of the two antibiotics almost coincide.

This 106-fold greater effectiveness of penicillin was, however, the maximum difference obtained in the present experiment. When the curves of the two antibiotics were plotted for a 90 min exposure time (see inset of figure 1), only the portions of the curves which corresponded to the lower concentrations were superimposable. Thus, although synnematin never equalled penicillin in activity on an absolute basis, after short exposure periods it was as much as $1/15$ as effective as penicillin.

That the relative effectiveness of synnematin is influenced by concentration as well as exposure time is evident from the fact that both the 90 min and the 265 min curves diverge at higher concentrations. Synnematin at concentrations of 3.42×10^{-2} μ moles per ml (9.6 units per ml) and above not only inhibited cell growth, but also reduced optical density (large graph, figure 1) to a value below that of the medium immediately after inoculation. Thus synnematin ranged in effectiveness from no inhibition of cell growth to destruction of the inoculum when the concentration was increased only 16-fold (from 4.27×10^{-3} to 3.42×10^{-2} μ moles per ml). Penicillin concentration was increased by 32-fold without achieving a similar range of activity.

Figure 2. Effect of synnematin pretreatment on penicillin binding. Cell samples with 0.07 g cell dry weight per sample were treated for 30 min with varying concentrations of nonradioactive synnematin in a final volume of 8 ml. Cells were then washed twice with water and were exposed for 20 min to 8.4×10^{-4} μ moles (0.5 units) of S^{35} -penicillin per ml. After three more washings, cells were assayed for S^{35} -penicillin binding.

It may be concluded from the data of the present experiment that synnematin is from $\frac{1}{15}$ to $\frac{1}{100}$ as active as penicillin against *S. aureus*, depending upon exposure time and concentration.

Binding of synnematin by *S. aureus*. The first data indicating that synnematin was bound by *S. aureus* was derived from experiments in which suspensions of resting cells were treated first with varying concentrations of nonradioactive synnematin B and then, after washing, with S^{35} -penicillin. As was shown by Pollock (1957) to be true of *Bacillus cereus* cells pretreated with cephalosporin N (synnematin B), pretreatment of *S. aureus* with synnematin B resulted in inhibition of S^{35} -penicillin binding (figure 2). Therefore, synnematin apparently reacted with at least one type of penicillin binding site. However, even when synnematin was present in a concentration greatly in excess of that shown in other experiments to be sufficient for complete synnematin binding within the

TABLE 1

Effect of repeated washing on synnematin and penicillin retention by *Staphylococcus aureus*

No. of Washings	Retained Antibiotic (μ moles/kg Dry Cell Wt)	
	Synnematin	Penicillin
0	9.05	15.5
1		9.81
2		9.04
3	5.87	8.32
4	5.13	8.80
5	6.69	8.80
6	4.49	

Cells (0.65 g dry wt) were exposed for 30 min to 20 ml of 1.99×10^{-3} μ moles S^{35} -synnematin per ml. Cells were then centrifuged and washed repeatedly with 40 ml portions of distilled water. After each washing (except 1 and 2) cells were analyzed for synnematin retention. The same procedure was followed with penicillin, except that cells were incubated for 15 min with 30 ml of 8.4×10^{-4} μ moles (0.5 units) of S^{35} -penicillin per ml.

given exposure time (0.5 hr), inhibition of penicillin binding was only 75 per cent complete.

The results above might be due to either or both of two causes. Penicillin may have been bound to sites not reactive to synnematin, or S^{35} -penicillin degradation products present in the penicillin solution may have been bound nonspecifically (Cooper, 1956). In either case synnematin would be unable to compete for all of the sites capable of binding S^{35} -labeled compounds. This is considered in more detail in the discussion of binding rates.

That synnematin was firmly bound by cells and was not removed by washing is shown by the data of table 1, which gives the synnematin content of cells treated with S^{35} -synnematin and then washed repeatedly with large volumes of water (15 volumes of water to 1 volume of wet cells). For comparison, results of a similar experiment done with S^{35} -penicillin are also included in the table.

It may be seen from table 1 that after three washings the synnematin content of cells remained at 5.55×10^{-3} (± 14 per cent) μ moles per g of dry cell weight. Penicillin content of cells after similar treatment was 8.74×10^{-3} (± 2.4 per cent) μ moles per g cells.

From this experiment it might be concluded

TABLE 2

Effect of incubating S³⁵-synnematin or S³⁵-penicillin cells with nonradioactive synnematin or penicillin

Binding Antibiotic	Incubating Solution	Retained S ³⁵ -Antibiotic (μmoles/kg Cells)				
		Incubation time (hr)				
		0	0.5	1.0	2.0	4.0
S ³⁵ -Synnematin	Phosphate buffer	3.92	3.98	3.34	3.20	2.67
	S ³² -Penicillin	3.92	4.63	3.98	2.95	2.46
	S ³² -Synnematin	3.92	3.56	3.02	2.88	2.28
S ³⁵ -Penicillin	Phosphate buffer	6.71	5.57	5.47	5.94	5.60
	S ³² -Penicillin	6.71	5.32	5.36	4.93	4.36
	S ³² -Synnematin	6.71	5.55	5.75	5.03	4.48

Cell samples (0.32 g dry wt per sample) were exposed for 15 min to 2.67×10^{-3} μmoles (0.75 units) S³⁵-synnematin per ml or for 10 min to 8.4×10^{-4} μmoles (0.5 units) S³⁵-penicillin per ml. After cells had been washed thoroughly with water, they were incubated with 20 ml of one of the following: nonradioactive synnematin or nonradioactive penicillin, dissolved in phosphate buffer, or phosphate buffer only. These reaction mixtures were incubated at 30 C in 25 by 200 mm test tubes on a rotary shaker. Samples were taken at intervals for antibiotic retention determinations.

TABLE 3

Effect of concentration on synnematin and penicillin binding

Antibiotic	Conc (μmoles/L)	Antibiotic Bound (μmoles/kg Cells)
Synnematin	0.445	4.59
	0.890	5.62
	1.78	7.91
	3.56	7.51
	7.12	7.97
Penicillin	0.210	7.81
	0.420	7.38
	0.840	8.94
	3.36	7.48
	6.72	8.61

Cell samples, each with 0.11 g cells (dry wt), were incubated with the indicated concentrations of S³⁵-synnematin or S³⁵-penicillin. μMoles of synnematin added per sample varied from 4.2×10^{-3} to 1.68×10^{-2} . Exposure to synnematin was for 30 min and to penicillin was for 15 min.

that somewhat less synnematin is bound by cells but that it is retained as well as penicillin.

The difference in amount of binding may not be significant, since the experiment was done to determine only if bound synnematin could be washed out with water and no particular effort was made to ensure complete binding before washing was begun. Also, the very low specific activity (about 5×10^5 cpm per μmole with a

flowing-gas counter) of the synnematin made radioactivity determinations subject to considerable probable error.

The firmness of synnematin binding was also shown by studies of the stability of the bound synnematin and penicillin complexes.

Stability of the bound synnematin and penicillin complexes. The effects of long exposure to buffer and nonradioactive penicillin or synnematin on bound S³⁵-penicillin and S³⁵-synnematin are shown by table 2. These data were obtained in experiments in which cells, treated first with S³⁵-synnematin or S³⁵-penicillin and then thoroughly washed, were incubated for 0 to 4 hr in pH 6.3 phosphate buffer or in buffer solutions of nonradioactive penicillin or synnematin.

It can be seen from table 2 that both S³⁵-penicillin and S³⁵-synnematin cells when incubated with phosphate buffer alone showed slightly less radioactivity loss after 4 hr than did cells incubated with unlabeled synnematin or penicillin. This effect was probably due to a small amount of equilibration of bound labeled antibiotic with free unlabeled antibiotic. However, bound synnematin seemed no more susceptible to replacement by either free synnematin or penicillin than did bound penicillin. This would indicate that the synnematin complex does not dissociate more readily than the penicillin complex.

Relation of amount of binding to antibiotic concentration. When cells were exposed to vary-

Figure 3. Synnematin uptake by *Staphylococcus aureus* with two sub-bacteriostatic concentrations of synnematin. Two cell samples (0.4 g cell dry weight per sample) were diluted to 53 and 160 ml. S^{35} -Synnematin was added to give concentrations of 4.28×10^{-4} and 1.43×10^{-4} μ moles per ml, respectively. The penicillin data were obtained by exposing a cell sample with 0.55 g cell dry weight to 500 ml of 1.68×10^{-5} μ moles (0.01 units) S^{35} -penicillin per ml. All samples were washed 3 times with large volumes of water before counting.

ing concentrations of S^{35} -synnematin or S^{35} -penicillin and washed three times with large volumes of water, the results given in table 3 were obtained. This table shows that the amount of synnematin bound by cells was independent of synnematin concentration, once a level of 1.8×10^{-3} μ moles per ml had been reached, and synnematin binding remained constant at 7.8×10^{-3} (± 2.4 per cent) μ moles per g cells. This indicates that synnematin, like penicillin, is bound specifically, so that once the binding sites are occupied further uptake does not occur, regardless of the amount of free antibiotic that is present.

The smaller amount of binding with 4.45 and 8.90×10^{-4} μ moles synnematin per ml was due to the length of the exposure period, which was shown by binding rate studies (described below) to be too short for completion of binding at these concentrations of synnematin. Since penicillin binding was complete within 15 min when the concentration of penicillin was only 2.10×10^{-4} μ moles per ml, the data of the present experiment

show not only that synnematin is bound specifically, as is penicillin, but also that synnematin is bound much more slowly than penicillin.

Rate of binding of synnematin and penicillin. Synnematin binding rate was studied by a method similar to that used by Maass and Johnson (1949b) in studying penicillin binding rate. Cell samples were exposed to S^{35} -synnematin solutions at concentrations of 1.43×10^{-4} and 4.28×10^{-4} μ moles per ml. Samples were taken at intervals and, after three washings with water, were analyzed for radioactivity. A parallel experiment was done with S^{35} -penicillin at 1.68×10^{-5} μ moles per ml.

Upon comparison of the results of the present experiments (given in figure 3) with those obtained by Maass and Johnson (1949b) from their work with penicillin, certain differences between synnematin and penicillin binding become apparent. Although the synnematin binding rate proved to be dependent on concentration, as reported for penicillin by Maass and Johnson, the binding rate was not found to be directly proportional to synnematin concentration, as

Figure 4. S^{35} -Penicillin binding in the presence of synnematin. Cell samples, each with 0.2 g cell dry weight, were incubated for 20 min in 14 ml portions of the indicated synnematin and S^{35} -penicillin mixtures. Penicillin concentration was 8.4×10^{-4} μ moles (0.5 units) per ml for all samples; synnematin concentration varied from 0 to 0.51 μ moles (143 units) per ml.

TABLE 4
Uptake of S³⁵-penicillin by cells pretreated with nonradioactive penicillin

Binding Antibiotic	Incubating Antibiotic	S ³⁵ -Penicillin Content (μmoles/kg Cells)				
		Incubation time (min)				
		0	44	75	119	239
S ³² -Penicillin	S ³⁵ -Penicillin	0	1.09	2.35	2.77	3.90
S ³⁵ -Penicillin	S ³² -Penicillin	5.21	4.88	5.80	4.88	4.37

Cell samples (0.6 g dry weight per sample) were exposed to S³²-penicillin (2.02×10^{-3} μmoles per ml) or S³⁵-penicillin (1.85×10^{-3} μmoles per ml) for 15 min. Both samples were washed with water. S³²-penicillin cells were then incubated with 1.85×10^{-3} μmoles S³⁵-penicillin per ml; S³⁵-penicillin cells were incubated with 2.02×10^{-3} μmoles S³²-penicillin per ml. Samples were taken at intervals and, after washing, were assayed for S³⁵-penicillin content.

was shown by the same workers to be true of penicillin. Also, in the present experiments penicillin was bound about 5 times faster than reported by Maass and Johnson. Reasons for these differences are not known.

When binding rates of synnematin and penicillin for the first 20 min exposure periods are calculated from the data of figure 3, it is found that penicillin is bound 28.5 to 69.0 times more rapidly, depending upon which synnematin curve is used for the comparison.

A similar difference in binding rate can be calculated from the data of experiments in which cells were exposed to mixtures of nonradioactive synnematin and radioactive penicillin. In such a situation the two antibiotics were in competition for binding sites, so that the number of sites occupied by an antibiotic depended upon its binding rate. In figure 4 are the results of one determination.

It is evident from figure 4 that synnematin gave penicillin poor competition for binding sites. Even at concentrations 600 times greater than that of the penicillin some penicillin was still bound. In fact, calculations based on these data indicate that the synnematin concentration could have been increased to infinity without completely eliminating penicillin binding. If it is assumed that two types of binding sites are present, one type available to penicillin and synnematin and the other available only to penicillin, it can be calculated (1) that the binding rate of penicillin is 70 times greater than that of synnematin when both are present at equimolar concentrations, and (2) that for every g of cells, 4.2×10^{-4} μmoles of penicillin were bound to sites not available to synnematin.

The binding rate ratio obtained through the

competition experiment agrees well with that obtained from the binding rate study. However, the value (5 per cent) for sites available only to penicillin is not in accord with the value (25 per cent) obtained by treating cells first with synnematin and then with penicillin (figure 2). That this discrepancy was due simply to differing amounts of nonspecific binding of S³⁵-penicillin degradation products seemed likely both in view of Cooper's findings (Cooper, 1956) and of the data given in table 4. In this experiment, cells were treated first with nonradioactive penicillin and then with S³⁵-penicillin, the reverse order to that used in the experiment of table 2. It was observed that uptake of radioactivity by cells was rapid and that after 4 hr as much radioactivity had been bound by these samples as by controls which had not been pretreated with unlabeled penicillin. Since, as shown by table 2, the loss of labeled antibiotic during the same time of exposure to unlabeled antibiotic was only about 18 per cent, penicillin or its degradation products were obviously reacting at sites other than those involved in specific binding.

Because the experiment mentioned above involved long exposure periods and high concentrations of incubating antibiotic, the results do not necessarily apply to the competition or inhibition experiments. Therefore, two other methods of estimating the amount of nonspecific binding were adopted. These are described below.

Determination A consisted of treating cells at 25 C for 0.5 hr with a penicillin solution in which 70 per cent of the total radioactivity was due to degraded penicillin. The amount of active penicillin present was less than that necessary to combine with all cell sites. This determination

TABLE 5
*Effect of S³⁵-penicillin degradation products
on S³⁵-binding*

Sample	Radioactivity			Radioactivity Due to Penicillin	
	Total added	In supernatant	In cells	Bacterial* assay	Chromatography†
	<i>cpm</i>	<i>cpm</i>	<i>cpm</i>	%	%
A	5710	4180	1550	26.5	29.5
B	5710	4320	1500	26.4	29.5

Cells (0.14 g cell dry weight per sample) were exposed for 30 min to less S³⁵-penicillin (5.0×10^{-4} μ moles) than was needed for complete binding. After treatment cells were washed with water. Original S³⁵-penicillin supernatant fluid and the first cell wash were combined. This solution and the washed cells were assayed for radioactivity. The percentage of added radioactivity that was bound by cells was compared with the chromatographically determined value for percentage of penicillin radioactivity in the stock solution.

* Percentage of total radioactivity bound by cells.

† Percentage of total radioactivity due to penicillin, according to chromatographic data.

TABLE 6
*Uptake of penicillin in the presence of its
degradation products*

S ³⁵ -Penicillin Conc (μ moles/ml)	Radioactivity Bound (cpm/g Cell Dry Wt)
50.3	13,675
100	13,580
201	13,290
419	13,475
839	12,580

To a series of cell samples, each with 0.023 g cell dry wt, were added solutions with the indicated S³⁵-concentrations. Seventy per cent of the radioactivity of the S³⁵-penicillin stock solution was due to penicillin degradation products. The amounts of penicillin added to samples was from 8.9×10^{-4} to 1.42×10^{-2} μ moles, which was more than was needed for complete binding. Exposure was for 30 min at 25 C. Cells were washed twice with water before transfer to gelatin for counting.

was based on the assumptions that (1) all penicillin is removed from the incubating solutions by the cells and (2) that nonspecific binding would be demonstrated by the binding of more radioactivity than was shown by chromatog-

raphy to be due to penicillin. That cells did not bind a greater amount of radioactivity than could be ascribed to penicillin is shown by table 5. Only 26.5 per cent of the total radioactivity of the penicillin solution was bound by cells, although 29.5 per cent was due to penicillin.

Determination B, described by Cooper (1956) is based upon the principle that nonspecific binding increases with increase in antibiotic concentration and that specific binding is independent of concentration once a certain level of penicillin has been reached. Therefore, nonspecific binding would be revealed by a continued increase in binding of radioactivity as concentration was increased. No evidence of such an effect appears in table 6, for penicillin binding remained constant at about 13,500 cpm per g cells when concentration was increased 20-fold. Essentially the same result is shown in table 3, which gives the effect of concentration of a purer penicillin product on the amount of penicillin bound. In this experiment the average amount of binding was 8.0×10^{-3} μ moles per g with the maximum deviation (11 per cent) occurring at an intermediate, rather than at a high concentration of penicillin.

Results of the two checks for nonspecific binding indicate that the binding which synnematin could not inhibit (figures 2 and 4) was not nonspecific binding of degraded penicillin. This in turn suggests that penicillin itself reacts at a site or sites not available to synnematin.

DISCUSSION

The findings of the present study offer at least a partial explanation for the poor antibiotic activity of synnematin against gram-positive bacteria. This work was begun on the theory that, if antibiotic activity were dependent upon binding, low activity could be due to all or any combination of three factors: small amount of binding, low rate of binding, and lability of the bound complex.

That formation of a readily dissociated complex was probably not responsible for the behavior of synnematin was shown by the experiments on complex stability. Synnematin, once bound, seemed to be retained as well as penicillin.

Also, differences in activity do not seem to be the result of differences in amount of synnematin and penicillin binding, since 7.8×10^{-3} μ moles

of synnematin and 8.0×10^{-3} μ moles of penicillin were bound per g of cells. Because of the low activity of the S^{35} -synnematin used for the binding determinations, the value for synnematin in binding may have an error of ± 20 per cent. However, since the determination of bacteriostatic level showed that penicillin was 15 to 106 times more active than synnematin, it seems that differences in amounts of binding could at most be only partly responsible for differences in biological activity.

The comparison of the binding rates of penicillin and synnematin brought out differences of sufficient magnitude to account for a considerable difference in biological activity. It was found that penicillin was bound 28.5 to 69 times faster than synnematin and was from 15 to 106 times as effective as synnematin against growing cells. However, because of the design of the present experiments, it cannot be established whether the bacteriostatic activity of synnematin is due to slow reaction with binding sites or to slow penetration of the bacterial cell wall, as was postulated by Hale *et al.* (1953) to explain the lesser activity of polar penicillins against gram-positive bacteria.

SUMMARY

Synnematin is bound by resting cells of *Staphylococcus aureus*. The binding resembles the reaction of penicillin with the same organism in that (1) the amount of binding is independent of concentration, (2) the bound synnematin cannot be removed by washing, (3) the bound synnematin does not exchange readily with either free synnematin or penicillin, and (4) binding involves at least one type of site which reacts with penicillin. Also, synnematin binding is roughly equal in amount to penicillin binding by *S. aureus*, for 7.8×10^{-3} μ moles of synnematin and 8.0×10^{-3} μ moles of penicillin are bound per g of cells.

Synnematin differs from penicillin in that the latter is bound 28.5 to 69 times faster. This might account in part for the 15 to 106 times greater effectiveness of penicillin against *S. aureus*.

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