block of the injected acetylcholine by hexamethonium was a block of the access of acetylcholine to the receptors, and not a block of the receptors themselves. Burn & Gibbons (1964) decided to test this, and did so by choosing bretylium which is chemically similar to acetylcholine. They used the Finkleman (1930) preparation of the rabbit ileum to discover whether hexamethonium would block the action of bretylium in abolishing the response to sympathetic stimulation. They found that it did, from which it followed that hexamethonium was blocking the access of bretylium to the receptors, and thus there was reason to think that hexamethonium also blocked the access of acetylcholine to the receptors.

In the course of these experiments, observations were made to find out if the tertiary compound pempidine, which is a ganglion-blocking agent having a chemical resemblance to nicotine, had any effect in blocking sympathetic nerve endings. When pempidine was added to the bath containing the Finkleman preparation of the rabbit ileum, the following result was consistently obtained. In a concentration of 5×10^{-5} g/ml, pempidine in the first 10 min acted like bretylium in causing a gradually increasing block of sympathetic stimulation. Then the blocking action stopped although not more than 50% complete, and during the next hour the block became less. In the next 2 h the block became complete. The observations appeared to indicate that at first pempidine reached the nicotinic receptors on which the sympathetic impulse acts and began to block them. Then further access of pempidine to these receptors was prevented by pempidine itself, due to "auto-inhibition".

To return to the experiments of Löffelholz, he found in some of his experiments on the perfused heart that the amount of noradrenaline released by sympathetic stimulation was very much increased during the infusion of acetylcholine. This occurred when the concentration of acetylcholine infused was $5{\cdot}5 \times 10^{-5}$M, less than the concentration used previously, and when it had been infused for 1 min only. He could not offer a satisfactory explanation for this, but I think it likely that the infused acetylcholine added its effect to that released by stimulation, to release, in turn, more noradrenaline. We know that acetylcholine is concerned in the release of noradrenaline in the isolated rabbit heart from the work of Huković (1966).

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The spectrophotometric determination of ampicillin in body fluids

Smith, De Grey & Patel (1967) described a specific spectrophotometric method for the determination of ampicillin in pharmaceutical preparations, based on the copper facilitated formation of the stable acid degradation products, for which the presence of the intact antibiotic molecule is essential.

We have now adapted the method to the assay of ampicillin in chicken blood, bile

Table 1. *Detection of concentrates of ampicillin in plasma and bile*

Ampicillin added (μg)	Concentration (μg/ml) found in plasma Spectrophotometric assay	Microbiological assay	Ampicillin added (μg)	Concentration (μg/ml) Spectrometric assay found in bile
31	30 (90%)	34 (103%)	250	232 (93%)
62	60 (97%)		500	488 (98%)
125	121 (97%)		1000	975 (97%)
125	125 (100%)		2000	2010 (101%)
250	239 (95%)	298 (118%)	3000	3135 (104%)
250	242 (97%)	298 (118%)		
250	230 (92%)	241 (96%)		
250	230 (92%)	270 (108%)		
250	234 (94%)	221 (88%)		
500	468 (94%)			
500	476 (95%)			

and urine; this enabled us to know the antibiotic concentrations in body fluids during the movement of ampicillin through the body.

Plasma assay. Plasma (0·2 ml) from heparinized blood is taken in a 1·5 ml centrifuge tube containing absolute ethanol (0·4 ml). After thorough mixing and centrifuging for 5 min at 4500 rev/min, clear supernatant (0·2 ml) is taken in a 5 ml tube with citrate buffer solution (0·8 ml; pH 5·2) containing copper (15 μg/ml) [98·5 parts of a buffer prepared by mixing citric acid (46·4 ml of 0·1M) and disodium hydrogen phosphate (53·6 ml of 0·2M), and copper sulphate pentahydrate (1·5 parts of a 0·393% solution)]. The tube, agitated at 120 strokes/min, is incubated at 75° for 30 min and then cooled in ice. The sample is taken in a 1·5 ml cuvette and read at 320 nm against the blank given by the residual non-incubated fraction of the supernatant.

Bile and urine assay. Bile or urine (0·1 ml) is taken in a 10 ml centrifuge tube containing trichloroacetic acid solution (0·1 ml; 20%), immediately mixed and then citrate buffer (4·8 ml; pH 5·8) containing copper (15 μg/ml) is added [98·5 parts of a buffer prepared by mixing citric acid (39·5 ml of a 0·1M) and disodium hydrogen phosphate (60·5 ml of 0·2M), and copper sulphate pentahydrate (1·5 parts of a 0·393% solution)]; the final pH is 5·2.

After thorough mixing and centrifugation at 4500 rev/min for 5 min, two similar portions of the supernatant are poured into two tubes, one of which is incubated as above. The procedure is then as described for plasma.

The data in Table 1 show that the spectrophotometric assay can detect with reliability plasma concentrations of ampicillin down to 30 μg/ml. The results are reproducible and are in good agreement with those recorded with microbiological assay (plate diffusion test; *Sarcina lutea*).

The main advantages of the method are the comparatively short time (about 1 h), and the small volume of plasma needed to make the assay.

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