potential (epp) was recorded with a microelectrode at the neuromuscular junction. After a delay from the moment of application of TTX the epp produced by motor nerve stimulation disappeared abruptly. But when TTX was applied to the last node of Ranvier the epp disappeared in steps as if impulses were occasionally able to jump one blocked node, to propagate in the terminals. TTX blocks action potentials and not electronically conducted potentials and so this is evidence that the action potential is necessary for transmitter release.

When TTX is applied to the neuromuscular junction itself its effects vary with the exact location of the applying pipette: Katz and Miledi describe an example of blockage of transmitter release occurring in three steps, probably because of successive blockage of the terminals derived from the three myelinated branches of the axon in their preparation. In another experiment, transmission was blocked by replacing calcium ions in the muscle bath with magnesium ions and recordings were made from microelectrodes 90μ proximal and distal to a TTX pipette, all in line on the same unmyelinated terminal. TTX release stopped end-plate potentials at the distal electrode, but did not affect those at the proximal electrode, provided that the pipette with TTX was sufficiently distant ($>50\mu$) from the myelinated end. When the TTX electrode was close ($<30\mu$) to the proximal recording electrode, proximal blockage occasionally occurred without distal blockage, as if the action potential were jumping across the local block and propagating to the end of the unmyelinated terminal.

These results, implying the necessity of a propagated action potential for transmitter release (at least at the frog sartorius junction), seem at variance with the earlier finding (Katz and Miledi, *J. Physiol.*, **167**, 8; 1967) that strong depolarization of the terminals could release transmitter and produce end-plate potentials as neuromuscular junctions blocked by TTX. But it seems most likely that this occurred when the applied potential change exceeded the normal action potential both in amplitude and duration. Katz and Miledi therefore suggest that "active propagation of the impulse along the synaptic terminal is not merely a device for increasing the safety margin of transmission, but is essential in order to achieve effective release of the transmitter".

ENZYMES

Enzymes to Order

from our Enzymology Correspondent

THE total chemical synthesis of ribonuclease hit the American newspapers last week almost as forcefully as the news, just over a year ago, of the synthesis *in vitro* of an infective phage chromosome by Kornberg, Goulian and Sinsheimer. As before, "making life in a test-tube" was the popular catchline, a phrase people will have to learn to live with in the years ahead. Both pieces of work are achievements of technique rather than idea: the methods used in making the enzyme are not new; they proved their worth for insulin some years ago. Still, ribonuclease, 124 amino-acids long, is more than twice as big as insulin, and the work certainly ranks as a technical *tour de force*.

Two groups synthesized the enzyme at about the same time: Dr Robert Merrifield and Dr Bernd Gutte of the Rockefeller University, New York, and Dr Robert Denkewalter and Dr Ralph Hirschmann of Merck, Sharp and Dohme, New Jersey. The two teams approached the problem in very different ways. The group at the Rockefeller University used a solid phase method developed a few years ago by Dr Merrifield himself. This method makes a passable analogy to biological protein synthesis, with polystyrene beads standing in for ribosomes. The C-terminal amino-acid of the intended peptide is anchored to its polystyrene bead by benzyl esterification, and the other amino-acids are added sequentially, their carboxyl groups activated in a standard way and their amino-groups protected by butyloxylcarbonyl. The cycle of coupling and deprotection can be programmed automatically, and Dr Merrifield's group apparently made the complete chain of ribonuclease, a process involving 369 separate chemical reactions, in the surprisingly short time of three weeks. The method has its adherents and its opponents in the world of protein chemistry: its chief drawback is that each coupling step must go to essential completion, better than 99 per cent, if the method is not to yield an unpurifiable mixture of permuted peptides.

The Merck approach was relatively conventional. The workers there synthesized several enzyme fragments by stepwise reaction of amino-acids in free solution, and finally assembled the fragments to make the complete enzyme. Amino-acids were applied in their N-carboxyanhydride form, a cyclic derivative which simultaneously activates the amino-acid's carboxyl group and protects its amino-group. The simple act of warming the reaction mixture exposes the amino-group, and prepares the growing peptide for the arrival of the next amino-acid. Unlike the solid phase synthesis, this method entails a purification step of some sort after each reaction step.

So, for the first time, a functional enzyme has been synthesized *de novo*. That the final product of these two teams turns out to be an active enzyme is a very pleasant confirmation of ideas about the primary structure of the molecule. It is perhaps doubtful whether man-made enzymes will, as has been claimed, make a swift impact on medicine, for ribonuclease is the smallest enzyme and living tissue, after all, is basically very cheap. Perhaps the new synthetic enzymes will made their mark quickest in the research laboratory: the ability to make families of molecules that are precisely tailored variants of an original enzyme will provide some extraordinarily sensitive tests for current ideas about enzyme mechanism.

What is the next enzyme likely to appear in a chemist's flask? Nobody has carried out a census of half-complete peptide chains in the world's laboratories recently, but it is known that Anfinsen is well advanced in the synthesis of micrococcal nuclease.

RNA PHAGES

Added End

from our Cell Biology Correspondent

ALTHOUGH rumour has it that Spiegelman's group is switching its attention from Qβ and the other RNA