



Diagram of possible reflex arcs concerned in muscle tonus.

planning future experiments. It assumes that tonic impulses coming from the spinal cord along the dorsal roots pass through synapses in the spinal ganglia and are relayed along the sensory fibers to the muscles. These are assumed to give off side branches with special endings on the muscle fibers. As a necessary corollary we must assume the possibility of axon-reflex-tonus. This would bring the tonic innervation of the muscles into the same category as the vasodilator innervation of the blood vessels.

It is possible that tetanus toxin may act by increasing the excitability of this axon-reflex-arc and this possibility we expect to test by removing the spinal ganglia and allowing the sensory fibers to degenerate before injecting the toxin.

### 3074

#### A test for bile salts in urine.

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A search through the text books of physiological chemistry reveals two tests that are used for the detection of bile salts in urine. One is the Pettenkofer reaction which depends on the production of a red coloration when a strong acid, furfural, and

bile salts are mixed. The other, the Hay test, depends on surface tension change due to the bile salts. Because of difficulty in eliminating the urinary pigments, the Pettenkofer test has not been adapted for use as a quantitative test. Recently Meyer<sup>1</sup> has devised a method for quantitating the bile salts in urine dependent on surface tension changes.

The following method can be used as a qualitative test with relatively simple apparatus. (For the quantitation of the bile salts a nephelometer is needed.) It is as follows: Thirty cc. of urine to be tested is mixed with thirty cc. of 95 per cent alcohol. One cc. of 25 per cent trichloroacetic acid is added together with a small quantity of charcoal. This mixture is boiled and then filtered. The charcoal remaining on the filter paper is washed with hot alcohol. The combined filtrate is made alkaline with sodium hydroxide and then evaporated to a volume of less than 15 cc. After cooling, the volume is made up to exactly 15 cc. and the fluid is then filtered. To five cc. of this slightly alkaline filtrate is added five cc. of normal sulfuric acid. To another five cc. is added five cc. of distilled water. After standing five minutes the tubes are compared in a beam of strong light. A cloudiness in the tube to which acid has been added indicates the presence of bile salts. The tube to which water was added should be clear.

In this method, as in the Pettenkofer test, the chief difficulty in quantitating the bile salts lies in the elimination of the urinary pigments. Charcoal removes these in part but seldom removes them completely. To overcome as far as possible this interference, we have taken a normal urine, added charcoal and rendered it acid. After boiling it is filtered. This urine is stored after autoclaving, in sterile flasks. In the process of autoclaving the urine becomes rather deeply colored. Dilutions of this sterile urine match fairly closely the tints obtained in the concentrated urine used in the test. The sterile urine solution is diluted until the color intensity of the two solutions is quite similar. A known quantity of sodium glycocholate is added to five cc. of this dilution. Five cc. of normal sulfuric acid is then added and the relative turbidity of the unknown and the standard is determined by reading in the nephelometer.

By this method as little as 2 mg. of sodium glycocholate per

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<sup>1</sup> Meyer, E., *Deutsch. Arch. f. Klinisch. Med.*, 1925, cxlvii, 274.

100 cc. of urine may be detected. Jaundiced urines examined have contained up to thirty mg. per 100 cc. Normal urine gives an entirely negative test.

The work with this test has not progressed sufficiently to allow any definite conclusions as to its clinical value. The presence of bilirubin in urine has long been used as an index of liver disease. It is known that certain degrees of liver damage can occur without the appearance of bilirubin in urine. In recent years the search is being made for more delicate methods of detecting liver injury. The bile salts are so peculiarly a product of the liver that they deserve a more careful study than has so far been given them.

## 3075

**The normal and abnormal response of amoeboid cells  
(amoebocytes of *limulus*) to stimulation.**

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Various considerations suggest very strongly the conclusion that the formation of acid within the amoebocytes is the factor directly responsible for the production of pseudopods and amoeboid movement. It seemed that it might be possible to obtain direct evidence bearing on this problem by allowing substances differing in their chemical and osmotic properties to act on the amoebocytes after the latter have migrated out of the piece of amoebocyte tissue into the surrounding blood plasma of *Limulus*. However, numerous experiments of this character proved that the amoebocytes respond to stimulation of various kinds with the sending out of normal or abnormal pseudopods and the consecutive movement of the granuloplasm into the latter. The response of these cells does, therefore, not primarily depend upon the character of the external stimulus, but upon the constitution of the cell which is such that it needs to react invariably with certain changes which when taking place in a definite sequence in