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The behavior of alanin in metabolism.By **A. I. RINGER** and **GRAHAM LUSK**.

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Injection of 20 grams of *i*-alanin in a completely phlorhizinized dog resulted in the elimination of "extra sugar" in the urine to an amount equalling 18.8 grams, or 93 per cent. of that theoretically possible. Although *i*-alanin is almost completely convertible into dextrose, preliminary respiration experiments indicate that it does not spare fat metabolism as effectually as does dextrose itself. This may be due to heat loss in the breaking down of alanin into simpler molecules (formic aldehyde?) and heat absorption in its construction into dextrose. A similar reasoning would serve to explain Rubner's "specific dynamic action" of protein.

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An important source of error in Heller's test for urinary protein.By **WILLIAM WEINBERGER**. (By invitation.)

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Heller's test for urinary protein is a fairly reliable one if care is taken in its application, but several urinary protein constituents give uncertain results with it. Thus, mucin fails to yield true precipitation — the "ring" is more or less opalescent and disappears on mixing. With nucleoalbumin the ring is not quite typical and is indistinct in undiluted urine. On the other hand various misleading factors, such as resinous acids, must be taken into account. Resinous acids may be ignored, however, if such products as *Balsamum Copaivæ*, or Santal Oil, have not been administered, or if the specific HCl test for resinous acids shows their absence. The turbidity formed with resinous acids dissolves on warming. The acids themselves may be removed by extraction with ether. In concentrated urine, as is well known, a uric acid ring may appear just above the line of junction of the urine and acid, and urea

may be precipitated in the form of glittering nitrate crystals ; but dilution of the urine prevents these effects.

In the course of our investigation of another problem, an additional source of error has been found in urines preserved with thymol in any of the usual ways. If Heller's test is applied to such urine after filtration, a ring will invariably appear even in the absence of protein. The ring is most marked, all other things being equal, in urines that have been treated with a *solution* of thymol, although it is very conspicuous in urines that have been preserved with powdered thymol.

The characteristics of this ring may be briefly stated as follows : A few seconds after the urine has been carefully poured upon the acid, there forms, precisely on the line of junction, a grayish white ring about 0.5 mm. high, resembling the albumin ring given by a faint trace of albumin, and gradually becoming more and more distinct, until, in some urines under conditions to be mentioned later, it presents the appearance of a heavy thick precipitate, the height of which increases continuously and renders the lower portion of the urine completely opaque. At this stage the color is somewhat different from that of a protein ring, in that it is more yellowish. Below the ring there is a greenish zone extending somewhat into the acid ; above it, a reddish zone smaller than the former and more contracted. The white ring is seen best in daylight reflected from a dark background, the color rings are seen best if the test-tube is held against a white surface.

On slightly disturbing the layers of urine and acid the ring, if a delicate one, disappears but reappears immediately. These effects can be obtained a few times in the same mixture. A *heavy* ring, however, will not completely disappear on slightly shaking but will gradually widen and extend into the upper urinary layer ; and it depends on the volume-relation between urine and acid whether complete mixing will remove the precipitate. On thoroughly shaking an excess of acid with little urine, a clear yellow solution results. If an excess of urine has been used, the mixture will not clear, but will remain turbid. Warming the mixture will not prevent the formation of this ring, nor will it clear the liquid, but, if anything, will make the reaction more distinct ; nor has dilution of the urine with three to four times its volume of water any marked effect.

This reaction is caused by the fact that thymol dissolves in urine when the latter is treated with it even in solid form, and it is noteworthy that more thymol is dissolved if the urine is neutral or alkaline, than when acid. Accordingly, an alkaline thymolized urine will give Heller's test more pronouncedly than a strongly acid one. But while the thick and heavy ring in the first case will have an appearance somewhat different from the protein ring and thus will hardly mislead one, the delicate thymol ring in the acid urine closely resembles a protein ring and is therefore more apt to cause confusion.

In urine containing both albumin and thymol in various amounts each ring may be discerned. The albumin ring is somewhat the wider of the two (about 2-3 mm.), and is whitish; whereas the thymol ring forms directly underneath and is grayer and thinner. The albumin ring may be completely covered by the thick thymol ring, so that the detection of protein may be seriously interfered with.

Further investigation proved that this ring is first formed by the precipitation of thymol by the concentrated acid. At this stage it closely resembles the albumin ring. Nitration of the thymol soon occurs, resulting in the formation of nitroso- and possibly nitro-thymol. This accounts for the gradual color-change from white to yellowish white, which the precipitate undergoes. A partially successful attempt has been made to isolate the nitro-substance or substances produced. With the aid of chloroform as the solvent, crystals of a yellowish brown color were obtained that gave Liebermann's nitroso reaction. These crystals melt at a temperature slightly above 50° C., whereas nitroso-thymol melts at 160° C. Thymol melts at 50°. It is quite probable, therefore, that very incomplete nitration occurred and that the brown crystalline product referred to was a mixture of thymol and a small amount of nitroso-thymol. Besides, some chloroform may have been occluded in the crystals. Work in this direction is proceeding.

To guard against this source of error, Heller's test cannot be directly applied to urine preserved with thymol. The latter must first be removed by extraction. Petroleum ether is very suitable for this purpose. Gentle agitation of the urine with an equal volume of petroleum ether in a test-tube for 2 minutes suffices to remove all traces of thymol.

When 5 grams of thymol were administered *per os* to a medium sized dog, the urine excreted during the succeeding 24 hours showed the familiar brownish yellow color. On standing it gradually became black. Heller's test was positive but, as the resultant precipitate had the same color as the urine, the exact significance of the result was uncertain. Thereupon different metabolic derivatives of thymol — thymo-sulfuric, thymo-hydrochinon sulfuric and thymo-glucuronic acids were isolated from the urine in the form of their chlorin substitution products. A small quantity of each of these substances was then individually added to *normal* urine, which in turn was subjected to Heller's test and invariably showed a positive reaction.

Agitation of such treated urine with petroleum ether in the manner above indicated did *not* extract thymol-glycuronic acid. This fact is of some importance; for while petroleum ether readily extracts thymol from urine to which thymol has been added as a preservative, it does not quantitatively extract from urine thymol that has been given internally and which is excreted in combined forms through the kidneys.

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A clamp for direct transfusion of blood.

A demonstration.

By **ISAAC LEVIN.**

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The clamp is similar in its construction to an artery forceps without the grooves. At the tip of each blade there is attached a small cannula with a smooth bore. At the inner edge of each cannula four small pin points are attached, and on the outer surface of the cannula four grooves are cut. When the clamp is closed, the pins of one cannula lie in the grooves of the other. The pins are bent outward and therefore the cannulas have a pyramidal form, so that each pin can lie snugly in its groove. At the beginning of the operation, both halves of the clamp are separated. The vein is pushed through one cannula and its wall is hooked on the pins. The same is done with the artery and the other half of the