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Imidazole Lactic Acid—A Component of Normal Human Urine.* (31711)

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In Europe, the only diazo-positive spot found after paper chromatography of 20 μl of urine is histidine(1); other spots would be considered abnormal. Recently a laboratory was established here for screening of non-human primate blood and urine by a variety of chromatographic and electrophoretic techniques, and the initial program included the training of technicians using their own urine and blood specimens. Following the application of a histidine-locating reagent to a paper chromatogram of urine specimens, one of the two examined showed a second red spot as well as a number of other red, gold and yellow spots. Subsequently 30 urines were obtained from laboratory staff and others and varying quantities of the second red spot were found in many but not all chromatogrammed specimens.

Materials and methods. Random urine specimens were obtained from 30 white and colored adults and infants; serum was obtained from all of the adults. All urines were desalted electrolytically(1) and one of the same urines was also desalted by ion-exchange. Urines were chromatographed on paper(1) and on thin layers of Avicel(2) but serum was run on paper(3) only. Butanol-acetic acid-water was used as the standard one-way solvent but other solvents and two-way procedures were also run to confirm identity of imidazoles. The DNPH-electrolytic reduction method(1) was carried out on a number of specimens to investigate the presence of imidazole pyruvic acid. Ethyl acetate extractions were made to distinguish between imidazoles which would be insoluble and phenolic acids and indoles, etc., which would be soluble in the reagent.

Results. A number of red and red-brown spots were found on urine chromatograms. The main component was histidine, with variable amounts of imidazole lactic acid and traces of other imidazoles. Parallel and co-chromatography with imidazole lactic acid confirmed the nature of the second imidazole compound. One of the trace components appeared to be imidazole propionic acid, although as the samples had been electrolytically desalted it might have been derived from urocanic acid; serum specimens showed none of the above compounds with occasional exceptions of traces of histidine.

Discussion. As judged from the spot area and intensity of color, the imidazole lactic acid spot was present in considerable although variable amounts in relation to the histidine concentration in a cross-section of the local population. A parallel study of over 200 non-human primates (to be reported later) of a variety of species including marmosets, baboons, capuchin monkeys, etc., showed a variable number of imidazoles to be present in the urine. Blood specimens from all species showed little or no histidine and no other spots when 8-10 μl were examined al-

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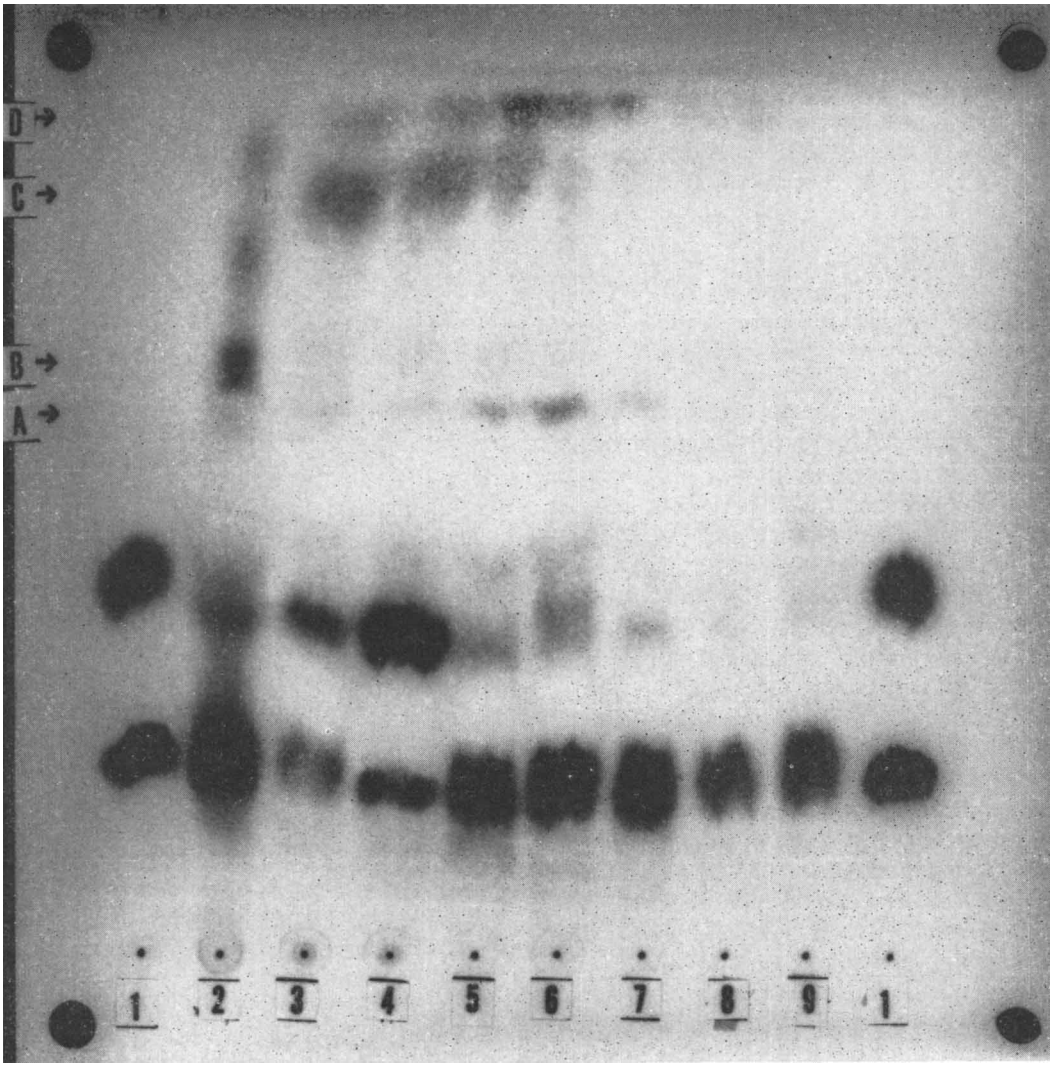

FIG. 1. Paper chromatogram of urines. 1, standard histidine plus imidazole lactic acid; 2, urine desalted by ion exchange; 3, urine desalted electrolytically; 4-9, urines desalted electrolytically; A, weak red spot; B, brown spot; C, golden yellow spot; D, yellow-brown spot. Samples 2 and 3 derived from the same urine specimen. Location reagent, diazotised sulfanilic acid; solvent, butanol-acetic acid-water.

though normal aminoacid patterns were always observed.

Young *et al*(4) investigated the diazotised-sulphanilic acid-positive spots found in human urine and showed a composite diagram which would be very similar to one which could be constructed from our results. This is useful confirmatory evidence of the existence of such spots on the chromatogram, particularly as the study was made in the same area about 200 miles from here. However, the conclusions they drew are now known to be erroneous and their studies were not pursued elsewhere. More recently Auerbach *et al*(5), in Philadelphia, investigated the urinary chromatograms of histidinemic children together with normal comparisons, in an apparatus similar to ours. Although not specifically stated, their results suggest that the only spot found in normal individuals' urine was histidine except after histidine-loading, when very small amounts of the lactate together with

other histidine metabolites showed up on the chromatograms. Our own studies on the urine of non-human primates from semi-tropical and tropical areas show a number of spots on the chromatograms with a suggestion of species differences. An interesting pattern has emerged from this study. Those urines obtained from individuals born in Europe and resident here only a short time showed the typical "European" pattern with histidine as the sole spot present, whereas urines from native Texans invariably showed the imidazole lactic acid spot as well as the histidine spot together with a variety of other reactors which varied from individual to individual without apparent pattern. Europeans who had been resident in this area for some years showed variable amounts of the lactate relative to the histidine level from almost nothing to the local levels. The time factor after which these substances began to appear is also highly individual as some respond almost within months while others show little imidazole lactic acid after many years local residence, although the other spots may be quite strong. In one case where the urine was also desalted by ion exchange procedures the imidazole lactic acid and histidine were again present in the correct chromatographic positions, whereas the pattern of other spots was different, suggesting either a chemical hydrogenation of an unsaturated compound during the electrolytic desalting or some other type of chemical conversion during the ion exchange procedure. It would be quite possible that the ammonia used to elute from the resin could bring about such a change, as, for example, when indolylacetyl glucuronide is converted to indolyl acetamide and indolyl pyruvate to a series of compounds which chromatogram as stable spots. This was not investigated further as these changes occurred in the non-imidazole components.

An explanation of this phenomenon remains to be found. It is known that a high daily intake of meat is reflected in increased excretion of methylhistidine and that fruits such as bananas, dates and certain berries can give rise to "abnormal" spots on urinary chromatograms but it does not seem feasible that dietary variation can explain this finding. The semi-tropical temperature plus humidity might well be implicated, particularly as imidazole lactic acid and other histidine metabolites are found in the urine of non-human primates living in similar tropical climates; although this might be a reflection of a different gut flora rather than a change in host metabolism.

Variations in phenolic acid patterns are well known, particularly when closed communities with unusual dietary or religious habits are compared with broader communities of much wider habits. Consequently, it is more than likely that other normal variations will be found. With the advent of mass screening techniques this phenomenon is worthy of notice, particularly as mass screening comes into wider use. It is apparent that the phenomenon can be considered as a normal variation. It is not reflected in blood patterns but may appear as major changes in the urine pattern. We are not able to undertake a geographical survey of this effect nor can we say whether it varies over the year.

Summary. Imidazole lactic acid has been found to be a normal component of urine in the Houston area. It gradually appears in the urine of those new to the area but in variable amounts.

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