by Dent[6]. 500 μl. of deproteinized and desalted plasma, and amounts of desalted urine corresponding to 250 μgm. of total nitrogen (Kjeldahl), were spotted on to 60-cm. squares of Whatman No. 4 paper, and were run in phenol saturated with water, followed by collidine–lutidine. The final positions of the amino-acids were revealed by spraying with a solution of 0·1 per cent ninhydrin in butanol, and examining after 24 hr. at room temperature.

Unidimensional chromatograms on Whatman No. 1 paper were run in butanol/acetic acid/water (4 : 1 : 5) using amounts of urine equivalent to 250 and 500 μgm. of nitrogen. No ornithine was detected after treatment with vanillin followed by alcoholic potash and heating. Histidine was detected in both specimens of urine by repeating the unidimensional runs in collidine–lutidine, and spraying with Pauly reagent.

In the first specimen of urine, cystine and lysine predominate, superimposed on a pattern consisting of glycine and taurine with traces of alanine, glutamine, serine, glutamic acid and methyl histidine. Traces of leucine and/or *iso*leucine and aspartic acid were obtained in the first specimen only, whereas threonine (confirmed by running with an authentic specimen) and β-alanine and/or citrulline were detected in both specimens of urine. Arginine was not detected in abnormal quantities.

In contrast to the affected dog, chromatography of a catheter specimen of urine obtained as a control from a healthy Labrador bitch nine months old revealed only a trace of cystine and no lysine. The amino-acid pattern consisted of glycine and taurine, with traces of alanine, glutamine, serine, methyl histidine and glutamic acid. The nitroprusside test for cystine was negative, and no cystine crystals were detected in the deposit after centrifuging the urine.

The amino-acid pattern of the plasma from the affected dog shows a small spot due to cystine (in contrast with the large spot found in the urine), supporting the view that we are dealing here, as in the human form of the disease, with a defect of tubular reabsorption of cystine from the glomerular ultrafiltrate. A similar mechanism would also account for the presence of lysine and threonine in the urine, since neither of these amino-acids was detected in the urine of the control animal.

The amino-acid pattern of the urine from this cystinuric dog is therefore, apart from threonine, identical with that found in cases of human cystinuria, while the finding of a low plasma-cystine points to a similar etiology.

Our thanks are due to Dr. Leon Duckett, who performed the surgery. One of us (C. W. C.) wishes to thank the Nuffield Foundation for a grant.

C. W. Crane*

Department of Biochemistry,
University of Melbourne.

A. W. Turner

Division of Animal Health and Production,
Commonwealth Scientific and
Industrial Research Organization,
Animal Health Research Laboratory,
Parkville, Victoria.

* Research Fellow in Chemical Pathology.

[1] Krabbe, A., *Vet. Rec.*, **61**, 751 (1944).
[2] White, E. G., *J. Comp. Path.*, **54**, 16 (1944).
[3] Blamey, E. R., and Brand, E., *Proc. Soc. Exp. Biol.*, **42**, 747 (1939).
[4] Uvarav, O., *Vet. Rec.*, **61**, 758 (1944) (discussion to ref. 1).
[5] Dent, C. E., and Rose, G. A., *Quart. J. Med.*, N.S., **20**, 205 (1951).
[6] Dent, C. E., "Paper Chromatography and Medicine", in "Recent Advances in Clinical Pathology", edited by S. C. Dyke (Churchill, London, 1951).

Formation of Calcium Superoxide

In connexion with an investigation on the formation and structure of certain alkaline-earth peroxides and superoxides, the dehydration of the compound $CaO_2.8H_2O$ has been studied. It has been proved that drying this hydrate with phosphorus pentoxide at room temperature will not yield pure CaO_2, but a substance with the following approximate composition: $Ca^{2+}(O_2^{2-})_{0.87}(O_2^-)_{0.05}(O^{2-})_{0.11}(H_2O)_{0.10}$.

The presence of superoxide ion (O_2^-) has been established by means of both chemical methods and by measuring the magnetic susceptibility.

The composition of this substance indicates that the dehydration is associated with a disproportionation of the following kind:

$$O_2^{2-} = (1-y)\,O_2^{2-} + \tfrac{2}{3}y\,O_2^- + \tfrac{2}{3}y\,O^{2-}$$

where y represents the degree to which the peroxide ions (O_2^{2-}) have been disproportionated.

Since the substance is hydrous, it is probable that the oxide ion exists as a hydroxide ion. Warming the dehydration product leads to an increase in the y-value, which, in the temperature interval 100–250° C., approaches 0·5. Heating to higher temperatures will cause decomposition.

X-ray photographs of the preparations have been taken using the Guinier method. In all cases the photographs exhibit only one phase with tetragonal symmetry. The a-axis is constant, but the c-axis increases slightly with increasing y. The following values have been obtained: $a = 5{\cdot}03$ A.; $c = 5{\cdot}95$–$5{\cdot}97$ A.

Intensity calculations and density determinations indicate that the phase concerned has a variable composition. It can be described as a compound CaO_2 with a CaC_2-structure where some of the O_2^{2-}-ions (peroxide ions) in statistical distribution are substituted by O_2^--ions (superoxide ions), O^{2-}-ions, and OH^--ions. Some of the Ca^{2+}-ions are also substituted by O^{2-}-ions.

The results obtained thus indicate that the composition of the phase can be described by the formula:

$$6(1-y)CaO_2 \cdot 2yCa(O_2)_2 \cdot 2yCaO \cdot 2yCa(OH)_2$$

where y ranges from $\sim 0{\cdot}1$ to 0·5.

This formula seems to be valid in all cases, where any warming has taken place of the product dehydrated at room temperature. Preparations obtained by dehydration at room temperature only are more hydrous than those which correspond to the general formula mentioned above.

If, on the other hand, the dehydration of $CaO_2.8H_2O$ is carried out directly at a temperature greater than 40° C., a phase is obtained the structure of which seems to be a superlattice of the tetragonal phase mentioned above.

These investigations, which also include corresponding compounds with other cations as well as peroxide-hydrates, will be described in detail in a forthcoming publication.

Cyrill Brosset
Nils-Gösta Vannerberg

Chalmers University of Technology and Research,
Institute of National Defence,
Gothenburg.
Sept. 13.

©1956 Nature Publishing Group