which would reduce citrate content by bacterial action was apparently not eliminated. Finally, a higher than normal excretion of calcium has been reported in a high percentage of patients with urinary calculi, a condition which might be expected to favor calcium precipitation.[5]

The present studies provide positive evidence for the existence of a renal mechanism by which the normal kidney can adjust the urinary excretion of citrate to the urinary output of calcium, and so alter the composition of the urine as to favor calcium solubility and, conversely, prevent stone formation. Suggestive evidence in support of this view-point was provided by two male subjects with uninfected urines, with a history of several recurrences of calcific renal stones, who were studied in a stone-free interval. Both showed a similar derangement of calcium-citrate metabolism on low calcium diets. Although the negative calcium balance fell within the normal range, abnormally large amounts of calcium were excreted in the urine and very little in the stool. The high urinary calcium output was accompanied by an unusually low content of citric acid as compared with normal subjects. One subject with bilateral renal calculi associated with hyperparathyroidism and a high urinary calcium, excreted abnormally small amounts of citrate even when urinary tract infection was largely controlled with sulfadiazine. On the other hand, the subject with hyperparathyroidism illustrated in Fig. 1, whose hypercalcuria was always associated with a high citrate excretion, has remained free of renal calculi over a period of almost two years of observation.

On the basis of these observations it is tentatively suggested that an impairment of the mechanism by which the kidney adjusts citric acid excretion to the urinary calcium output may be involved in the production of metabolic calcium urinary calculi by providing an environment favorable to the precipitation of calcium stones. In some patients with renal stones, this derangement is also accompanied by an unusually high urinary excretion of calcium, providing an even more favorable environment for stone formation. The factor of infection can further predispose towards stone formation by reducing the citrate content of the urine through bacterial action. Whether this postulated renal function operates through variations in citrate resorption or citrate production from precursors is now under investigation.

This concept provides a rational basis for a therapeutic regime designed to compensate for an impairment in the renal citric acid mechanism. Studies now in progress indicate that it is possible to increase urinary citrate excretion significantly without increasing the calcium output, by feeding various citric acid precursors of the C_4-dicarboxylic acid series. This procedure is free from certain theoretical and practical disadvantages attendant on the prolonged administration of alkalies or estrogens, which also increase urinary citrates.[1] A therapeutic regime of this character may be expected to help prevent recurrences of renal calculi and aid in the solution of those already present, as well as reduce the incidence of renal stones in the immobilized fracture case with hypercalcinuria.

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NEGATIVE WATER BALANCES DURING EXPOSURES TO LOW BAROMETRIC PRESSURES

RATS exposed to pressures of 380 mm Hg lose weight rapidly in comparison with rats kept at 760 mm Hg pressure but otherwise under identical conditions (fasted, water *ad lib*). When the composition of this weight loss was examined, it was found that the increased weight loss of the experimental animals was due to an increase in insensible water loss without a concomitant thirst. Table 1 shows typical results: 8 rats in each group were exposed for 6 hours to the noted pressures. The differences between the averages of the two groups with respect to loss of feces and urine and to water drunk are insignificant. But the insensible water loss (as measured by appropriate absorption methods) is doubled for the animals exposed to low pressures. From these measurements, a considerable negative water balance is evident. In spite of this loss of water from the organism, it should be emphasized, the water intake is not increased. The same phenomenon, with its apparent lack of compensating thirst, has been found to occur during exposures of 12, 18 and 23 hours.

TABLE 1

	Initial weight g	Weight loss g/100g	Feces g/100g	Urine cc/100g	Water drunk cc/100g	Insensible water loss cc/100g	Water balance cc/100g
760	164.2	4.8	0.4	1.0	0.5	2.5	−3.0
380	154.1	6.7	0.9	1.7	0.7	4.9	−5.9

Of the many factors operative at high altitudes which might be responsible for this negative water balance, anoxia appears to be the main causative factor. This is indicated by the two following experimentally established facts: first, the phenomenon is

prevented by giving oxygen instead of air to breathe, at pressures of 380 and 190 mm Hg; and, second, the phenomenon occurs when the rats are given, at 760 mm Hg pressure, a mixture of 10½ per cent. O_2 and 89½ per cent. N_2 to breathe. These two experiments also show that pressure changes *per se* are not responsible for the negative water balances. Furthermore, it is probable that anoxia causes the phenomenon because of the hyperventilation which it induces. When rats were made to hyperventilate by giving them gas mixtures high in CO_2, keeping the O_2 constant at 21 per cent., a negative water balance of considerable magnitude is induced. Thus, with 5 per cent. CO_2, it was 5.1 cc per 100 gms of rat; with 10 per cent. CO_2, 4.7 cc and with 15 per cent. CO_2, 5.2 cc. These figures approach, but do not quite reach, the negative water balance of exposure to 380 mm Hg pressure (5.9 cc; see Table 1). Hyperventilation in effect passes more air over the evaporating surface of the lung and there results a greater water loss.

We feel that this negative water balance may be of importance in the etiology of pilot fatigue. The latter is postulated by Armstrong[1] to be closely related to adrenocortical insufficiency. In our opinion, the chain of events leading to this insufficiency is as follows: first, a water loss, as demonstrated here, and, second, a renal salt loss, due to the previous water loss[2] and to the relative alkalosis of acapnia.[3,4] Such a salt loss has been shown to occur in mountain climbers[5,6] and during exposures to low oxygen tensions.[7] It appears to be in some way mediated by the adrenal cortex.[7] The combined salt and water loss, unaccompanied by thirst, puts a considerable strain on the adrenals; if repetitive, it would tend to produce a subacute adrenocortical insufficiency. McCance[8] has described a somewhat similar situation in which great loss of body salt and water was unaccompanied by thirst. This resulted, in his experiment, in a condition simulating adrenal insufficiency and in a train of symptoms startlingly like those of pilot fatigue.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

DETERGENTS AND STAINING OF BACTERIA

IN order to obtain a satisfactory microscopic preparation of bacteria, in which cells are uniformly distributed, it is necessary to have slides which are thoroughly cleansed by chemical methods, flaming, or both. Otherwise, the high surface tension caused by the presence of fat-like substances on the surface of the slides produces an uneven and massed distribution of the bacteria.

In course of the routine laboratory work it has been found that satisfactory preparations could be made on slides which were cleaned mechanically from the dust particles with a piece of cloth, if to the suspension of the bacteria a small amount of a detergent was added. The following procedure gave satisfactory results.

Water solutions of "Aerosol OT" or a saline solution of "Aerosol MA"[1] 1:500 were kept at hand. One loopful of one of those dilutions was placed with a loop on the slide. Bacteria were added from the liquid or solid media to this drop of detergent and the suspension was spread uniformly over the desired area with the loop. Preparations were air dried without heating, fixed with methyl alcohol or heat, and stained in the usual way. No detrimental effect of the presence of the detergent on the quality of the staining was noticed.

It was also found that the preparations made for the staining of the flagella gave the same results on the slides which were cleansed mechanically and the bacteria were suspended in distilled water containing "Aerosol OT" in the dilution 1:1000, as on the slides which were cleansed chemically and flamed with the bacteria suspended in distilled water alone. The quality of the flagella preparations was still better, however, when washed and flamed slides were used and the bacteria were suspended in the "Aerosol OT" solution. In such preparations the distribution of bacteria on the slides and the arrangement of the flagella were found most satisfactory.

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