subjected to a much more extensive Zn^{65} analysis. The Zn^{65} concentrations found in the various organs of this animal are listed in Table 2. It may be noted that the Zn^{65} concentration in the flesh, fat, and bone samples are about twice those observed in the samples of the previous year (see Table 1). It is also apparent that hair, liver, and bone concentrate zinc to a greater extent than the other organs.

The observation of these Zn^{65} concentrations in farm-produce samples suggested the possibility that Zn^{65} could be measured in individuals obtaining their food supply from these irrigation projects. For the measurement of Zn^{65} in people, a 3- by 5-in. sodium iodide crystal detector operated in a shielded room similar to the installation developed at Argonne National Laboratory (*2*) was used. The gamma-ray spectrum of an individual who consumed approximately 0.1 and 0.7 kg, respectively, of the meat and milk per day from the sources listed in Table 1 is shown in Fig. 1. This Zn^{65} photopeak area represents about 3.6×10^{-2} μc or 80,000 disintegration/min.

Twelve other individuals were measured whose diet did not include food from the irrigation project but did in some cases include drinking water whose source was the Columbia River. The gamma-ray spectra of most of the individuals whose drinking water originated in the Columbia River show a small Zn^{65} photopeak. The Zn^{65} content of these individuals was estimated to be between 5000 and 10,000 disintegration/min. The gamma spectra of individuals who receive neither their water nor their food supply from the Columbia River or its irrigation projects showed no detectable Zn^{65} photopeaks. The Zn^{65} found in all samples of foods, in fodder, or in individuals could be traced either to Columbia River drinking water or to food from the lower irrigation projects. It is believed that, with a sensitive total-body counting device of the Argonne National Laboratory type (*2*) Zn^{65} could be measured in most individuals who receive their drinking water from the Columbia River downstream from Hanford, provided that some purification step in the local water treatment does not remove the isotope.

Zinc-65 has been reported (*3*) in individuals living near the Pacific Proving Grounds and was shown to be a result of contamination from nuclear tests; however, Zn^{65} from nuclear tests has not been observed in foods raised in this country. The presence of Zn^{65} in the amounts observed here in no way constitutes a hazard. Even the value of 3.6×10^{-2} μc of Zn^{65} in the individual whose diet included the meat, milk, and drinking water of Table 1, is less than 0.01 percent of the total permissible body burden for this isotope (*4*). Also, even the highest Zn^{65} value in Table 1 (for pasture grass) is less than 5 percent of the maximum permissible concentration for human foods (*4*).

Smaller amounts of the radioisotopes Cr^{51} and Sc^{46} have been detected in samples of pasture grass but have not been found in farm produce.

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References

1. J. J. Davis and R. W. Perkins, unpublished.
2. C. E. Miller *et al.*, *Nucleonics* **14**, No. 4, 40 (1956).
3. ———, *Argonne National Laboratory Rept. No. ANL-5755* (July 1957), pp. 53–56.
4. "Recommendation of the International Commission on Radiological Protection," *Brit. J. Radiol. Suppl. No. 6* (1955).

23 September 1958

Studies on 4-Keto-L-Proline

Abstract. The administration of ketoproline to chick embryos resulted in an increase in the free hydroxyproline. This phenomenon is explained by the inhibitory action of ketoproline on the catabolism of hydroxyproline as well as by the conversion of the former to the latter. Ketoproline was found to be reduced to hydroxyproline by the supernatant fraction of rat-kidney homogenate in the presence of a reduced pyridine nucleotide.

Since hydroxyproline occurs uniquely in collagen in the animal, studies on the biogenesis and metabolism of this compound have a great significance in the understanding of the biochemistry of collagenous tissues. In the course of a survey of compounds structurally related to hydroxyproline for their ability to affect the metabolism of this imino acid, the administration of 4-keto-L-proline (*1*) to chick embryos was found to produce an increase in free hydroxyproline.

For these studies 5 mg of free 4-keto-L-proline in 0.2 ml of water was placed in the air space of a 12-day-old chick embryo through an opening in the shell. After 24 hours an 80-percent ethanol extract was prepared from the whole embryo and hydroxyproline was assayed colorimetrically by a modification (*2*) of the method of Neuman and Logan (*3*). The results are shown in Table 1. It can be seen that ketoproline caused a five- to sixfold increase in free hydroxyproline without affecting the proline level. Similarly, when 15 mg of ketoproline was administered subcutaneously to rats (200 to 250 g), a prolonged elevation of the blood hydroxyproline level was observed, an increase from 0.55 to 1.0 mg per 100 ml being maintained for a period of several hours. Although administration of 5 mg of hydroxyproline produced a comparable increase, this lasted for less than 2 hours. The mechanism by which ketoproline produces the increase in free hydroxyproline in vivo was found to be twofold: (i) inhibition of hydroxyproline destruction and (ii) conversion of ketoproline to hydroxyproline.

A strain of *Achromobacter* grown on hydroxyproline as the sole source of carbon metabolized hydroxyproline extensively. However, when increasing amounts of ketoproline were added to the incubation mixture, destruction of hydroxyproline was diminished (Table 2). Proline was also found to antagonize the metabolism of hydroxyproline under similar conditions. However, the metabolism of proline by proline-adapted *Achromobacter* was not appreciably affected by ketoproline. Similar observations were made when mammalian liver or kidney mitochondria were used in place of the bacteria. Neither the bacteria nor the mitochondria were able to convert ketoproline to hydroxyproline in any detectable amounts.

When ketoproline was incubated with a well-dialyzed soluble fraction of rat-kidney homogenate, it was reduced to

Table 1. Effect of ketoproline on free proline and hydroxyproline levels in chick embryos. Twelve-day-old embryos received either 0.2 ml of saline or 5 mg of ketoproline in 0.2 ml of water. After 24 hours, 80-percent ethanol extracts of the embryos were assayed for hydroxyproline (*3*) and proline (*6*).

Treatment	Wet wt. of embryo (g)	Free hydroxyproline (μg)	Free proline (μg)	Free hydroxyproline/proline free
Control (av. of 6)	5.2	112.1	206.2	1.84
Ketoproline administered (av. of 4)	6.3	627.2	218.7	0.35

Table 2. Effect of increasing concentrations of ketoproline on hydroxyproline metabolism by *Achromobacter*. One milligram of hydroxyproline was incubated for 30 minutes with 9.4 mg (dry weight) of hydroxyproline-adapted *Achromobacter* (*7*).

Ketoproline/hydroxyproline	Hydroxyproline remaining (μg)	Inhibition (%)
0	97	
1	340	29
2	420	36
3	585	54
4	690	66
5	780	76

Table 3. Enzymatic reduction of ketoproline to hydroxyproline. One milliliter of dialyzed supernatant fraction from a 1:2 KCl homogenate of rat kidney was used. All of the incubation beakers contained 0.5 ml of 0.5*M* phosphate buffer, *p*H 7.4; 1 mg of ketoproline; 5 μmole of nicotinamide, and 1 ml of rat-kidney preparation in a final volume of 3 ml. Where indicated, 0.5 μmole of DPN, 0.26 μmole of TPN, 200 μmole of glucose, and 250 units of glucose dehydrogenase were added. After 1.5 hours of incubation, hydroxyproline was assayed by a modification of the Wiss method (*2, 8*).

System	Hydroxyproline (μg)
DPN + glucose dehydrogenase system	42.7
TPN + glucose dehydrogenase system	52.5
DPN or TPN without glucose dehydrogenase	< 3.5

hydroxyproline. This reaction was found to require the presence of reduced pyridine nucleotides, either as such or generated in the incubation mixture by the glucose dehydrogenase system (*4*), as shown in Table 3. Reduced TPN (*5*) was found to be more active than reduced DPN. The rat-kidney preparation could not be replaced by purified commercial alcohol or lactic dehydrogenases. Neither reduced DPN nor reduced TPN was effective in the absence of the rat-kidney preparation.

The inhibitory effect of ketoproline on hydroxyproline metabolism is clearly established in these studies. The enzyme responsible for the reduction of ketoproline and the physiological significance of this reaction are under investigation.

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References and Notes

1. A. A. Patchett and B. Witkop, *J. Am. Chem. Soc.* 79, 185 (1957).
2. A report on the modified procedure is in preparation.
3. R. E. Neuman and M. A. Logan, *J. Biol. Chem.* 184, 299 (1950).
4. H. J. Strecker and S. Korkes, *ibid.* 196, 769 (1952).
5. The following abbreviations are used: DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide.
6. W. Troll and J. Lindsley, *J. Biol. Chem.* 215, 655 (1955).
7. We are indebted to Dr. R. N. Doetsch of the University of Maryland for this strain.
8. O. Wiss, *Helv. Chim. Acta* 32, 149 (1949).

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Direct Observation of Evaporation from Quiescent Water

Abstract. The color change of a filter paper impregnated with cobaltous chloride and held just above the surface of water gives a good indication of the rate at which evaporation proceeds from individual regions of the surface. The marked effect of some monolayers on thermal convection currents within the liquid can be thus shown.

The usual technique of measuring the rate of evaporation of water from a quiescent surface and the effect of monolayers upon this is rather elaborate (*1*) yet does not provide any information about local conditions over small portions of the area studied. This report (*2*) presents a few observations based on a simple technique which gives qualitative but very direct visual information about the rate of evaporation and shows what happens over areas of the order of a few square millimeters. The technique is based on the color change produced by the vapor reaching a sheet of paper impregnated with cobaltous chloride and held very close to the surface.

Figure 1 shows the pattern—which was actually pink on blue—obtained when the indicator paper was placed above a square cell, 2 by 2 cm, filled with water whose surface was divided into two parts by a polyethylene barrier. To the left of the barrier the surface was clean, while some cetyl alcohol was sprinkled over the surface to the right of the barrier. In the photograph, taken 2 minutes after the paper was placed above the surface, the difference in the rates of evaporation from the two sides is strikingly apparent. Over the clean surface the paper is already pink, while over the monolayer it is still largely blue. In addition, the color change over the clean surface is uniform (it developed uniformly from the beginning), while over the protected part the change appears in spots, which gradually spread over the whole area.

Similar irregular development of the color, signifying uneven rate of evaporation in the presence of the monolayer, was observed with a variety of vessels. It is attributed to the presence of relatively large convection currents which rise warm, cause relatively rapid evaporation, and are thus cooled so that the rate of evaporation is reduced while they continue along the surface for a distance before finally sinking. On a clean surface the convection pattern is different, and local differences are much smaller. This interpretation is supported by observation of convection currents made visible by very slow injection of a very dilute solution of fluorescein into the surface. The convection currents, while irregular, seem to be more extended in the presence of the monolayer, and their general pattern corresponds to that of the spots on the indicator paper. Changes in the thermal resistance of the water, reported previously (*3*), are also in agreement with this observation.

This change in convection currents is connected with the well-known hysteretic resistance of a monolayer against extensions and contraction (*4*). When a cooled streamline of water detaches itself from the surface and sinks under the influence of gravity, the corresponding surface must shrink. Conversely, when a rising warm streamline reaches the surface it causes, necessarily, a local expansion of the surface. When the surface is clean, such expansions and contractions encounter no resistance, but when a monolayer is present they are impeded, and convection at the surface develops only over greater distances and when larger forces are present. The heat transport to the surface from the bulk of the water is thus necessarily affected and localized.

In the experiments under discussion (*5*), the filter paper was firmly attached to a glass plate, both to insure an even surface and to prevent access of vapor from the back. The plate was first covered with a thin layer of "rubber cement for pasting paper," and the filter paper was firmly pressed onto it. The whole was then submerged in a moderately concentrated solution of $CoCl_2$ (prepared without heating), excess liquid was pressed out between filter papers, and the assembly was dried in a vacuum desiccator. The rims of the vessels had to be coated with paraffin to prevent creeping of the water. After the rim had been adjusted to the horizontal, the vessel was filled with water to within about 1 mm of the top. The water surface was protected, when protection was desired, by manual sprinkling of a few specks of commercial cetyl alcohol upon it. A wait of a few minutes allowed the convection currents to develop and stabilize. The glass-backed indicator paper was then

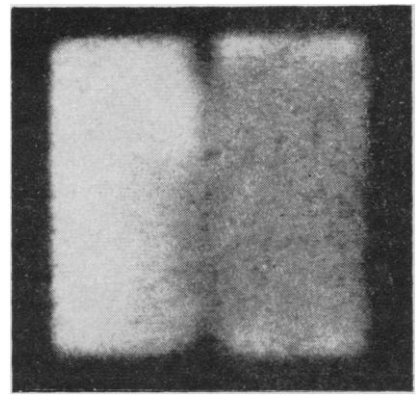

Fig. 1. The local pattern of evaporation from a water surface 2 cm square; the left side is clean, the right side is protected by cetyl alcohol. This photograph of cobaltous chloride indicator paper was taken 2 minutes after the paper was placed above the water surface.