tory effect caused a deficit of 10 impulses before the application of alcohol; of 1 impulse when the alcohol had exerted its effect; and of 11 impulses after recovery. The initial frequency in the last record was higher than in the first, presumably owing to further dark adaptation during the experiment, yet the number of impulses lost during inhibition was approximately the same. This is in accordance with Hartline's finding (2) that the number of impulses lost during inhibition is independent of the initial frequency over a wide range.

A single preliminary experiment has been made to determine whether three other substances have a selective effect on inhibition. Acetyl choline (100 mg/ 100 cm<sup>3</sup>) and curare  $(5 \text{ mg}/100 \text{ cm}^3)$ had no effect whatever. Nicotine (0.16 percent) caused spontaneous activity and then blocked conduction in the nerve fibers but had no selective effect on inhibition.

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# Fat Changes during Weight Loss

In the course of nutritional studies, 13 clinically healthy and active young white males were placed upon a low-calory diet yielding approximately 1000 cal/day for a period of 24 days (1). Soft-tissue teleoroentgenograms were taken at six anatomical sites during the preliminary control period and again at the end of the period of caloric restriction. These x-rays, made and measured under standard conditions (2), provided an accurate measure of changes in the subcutaneous fatplus-skin layer on nine parts of the body.

All of the subjects lost weight, while incurring a deficit of the order of 40,000 cal: the median weight loss was 8.3 kg, or 12 percent of the original value. Subcutaneous fat decreased with median losses of 4 to 5 mm for "central" fat (deltoid pocket, iliac, and trochanteric) and 1 to 2 mm for "peripheral" (lower arm and lower leg), as is shown in Table 1. Decreases in subcutaneous fat ranged

Table 1. Median values for subcutaneous fat and weight before and after weight reduction and changes in fat per kilogram of weight loss.

Median (before)	Median (after)	Decrease (median)	Decrease (per kg)
69.1	60.7	8.3	
4.4	3.4	1.0	0.1
3.8	2.7	0.7	0.1
12.5	7.7	4.4	0.5
12.2	7.1	5.7	0.7
13.6	9.5	5.9	0.6
5.6	4.7	0.9	0.1
3.0	2.7	1.1	0.2
76	5.7	2.3	0.3
5.9	4.3	1.5	0.2
	(before) 69.1 4.4 3.8 12.5 12.2 13.6 5.6 3.0 7.6	(before)         (after)           69.1         60.7           4.4         3.4           3.8         2.7           12.5         7.7           12.2         7.1           13.6         9.5           5.6         4.7           3.0         2.7           7.6         5.7	(before)(after)(median)69.160.78.34.43.41.03.82.70.712.57.74.412.27.15.713.69.55.95.64.70.93.02.71.17.65.72.3

from 16 percent to 47 percent of the initial values. The rate of fat lost per kilogram of weight loss ranged from 0.1 to 0.6 mm, depending on the part considered.

Losses in subcutaneous fat were clearly related to the initial thicknesses. Those parts of the body with the thickest fat deposits sustained the greatest loss dur-. ing caloric restriction (Fig. 1, top). In like fashion, those individuals with greater amounts of fat to start with sustained greater losses in fat (Fig. 1, bot-



Fig. 1. Relationship between initial subcutaneous fat thicknesses and amount of subcutaneous fat lost after 24 days on a 1000-cal diet. (Top) Scattergram showing changes in the nine fat thicknesses measured in this study. (Bottom) Scattergram showing changes in 13 subjects. [Individual mean Z scores for fat are used as an indication of total fatness (4).]

tom). Rank-order correlations in each case were found to be highly significant, an exact test for significance (3) being used.

Since fat is withdrawn in proportion to the initial amount of fat present, relative fat patterns before and after weight reduction tend to preserve their individual characteristics. This finding has been published elsewhere (4).

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### **References** and Notes

- 1. This paper reports research undertaken in co-operation with the U.S. Quartermaster Food and Container Institute and has been assigned No. 543 in the series of papers approved for publication. The views or conclusions contained in this report are our own. They are not to be construed as necessarily reflecting the views or endorsement of the U. S. Department of Defense
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**Reaction of 8-Quinolinol** with Cerium (III)

## The reaction between cerium (III) and 8-quinolinol was first studied by Pirtea (1), who used it for the gravimetric determination of this ion. The unusual nature of this reaction was indicated when it was found that the formula of the precipitated chelate was $Ce(C_9H_6NO)_4$ · $2H_2O$ , containing 18.73 percent cerium. The cerium had not been precipitated as the trivalent chelate, but an oxidation had occurred giving a chelate in which the cerium was present in an oxidation state of four. Although 8-quinolinol usually acts as a reducing agent, in this case it was the oxidizing agent.

Berg and Becker (2) found that the precipitation of cerium (III) by 8-quinolinol from a tartrate-buffered solution gave the trivalent chelate corresponding to the formula  $Ce(C_9H_6NO)_3$ . Identical results were obtained using the method of homogeneous precipitation (3). In both of these cases the cerium was present in the trivalent state in the chelate.

Since there is a difference in color of the two chelates—the trivalent chelate is yellow, whereas the tetravalent chelate is purple—it was thought that a study of the absorption spectra of the chelates might be useful as an analytic method. A method may be developed for the determination of cerium in a mixture with other rare-earth metal ions, since the other rare-earth chelates with 8-quinolinol are all yellow in color (3). Also, a study of the absorption spectra might be helpful in the elucidation of the structure of the tetravalent chelate.

The absorption spectra of  $Ce(C_9H_6)$ NO)<sub>4</sub> in  $\overline{CHCl_3}$  and 1N HCl and  $Ce(C_9H_6NO)_3$  in 1N HCl are shown in Fig. 1. The curves were determined with a Beckman, model DU, spectrophotometer, using 1.00-cm silica cells. The chelates were prepared by methods already described (1, 2). The absorption spectra of the two chelates in 1NHCl are identical, giving maxima at 312 and 360 mu. 8-Quinolinol also gives identical maxima in 1N HCl, which indicates that the two chelates are completely dissociated in this solvent. This is what would be expected from the ready solubility of the chelates in strong acid solution.

The spectrum of the cerium (IV) chelate in CHCl<sub>3</sub> exhibits three maxima in the wavelength region studied. Max-



Fig. 1. The absorption spectra of the cerium 8-quinolinol chelates in various solvents. (A)  $Ce(C_{9}H_{6}NO)_{4}, 3.79 \times 10^{4}M$  in CHCl<sub>5</sub>; (B)  $Ce(C_{9}H_{6}NO)_{4}, 1.72 \times 10^{4}M$  in 1N HCl; (C)  $Ce(C_{9}H_{6}NO)_{5}, 2.18 \times 10^{4}M$  in 1N HCl.

ima were observed at 307, 370, and 480 mµ. Obviously, the 307- and 370-mu maxima are those of 8-quinolinol, but the 480-mµ maximum is caused by the chelate itself. This would indicate that the tetravalent chelate could not be an addition product having the composition  $Ce(C_9H_6NO)_3 \cdot C_9H_6NOH$ . The trivalent chelate was too insoluble to be studied in CHCl<sub>3</sub>.

The maximum at  $480 \text{ m}\mu$  could thus be used for the qualitative or quantitative determination of cerium, either alone or in the presence of other rare-earth metal ions. Further studies are being conducted on this determination.

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13 August 1956

## X-ray Microscopy of Thin Tissue Sections

The methods of projection x-ray microscopy have now been developed to the point where applications can be sought in a wide variety of problems. In the field of biology one of these is the examination of sections of tissue. Thus, it will be instructive to view relatively thick slices stereoscopically and also to compare the appearance of thin sections under the optical and x-ray microscopes. For such comparisons the sections must be no more than a few microns thick, as indeed they must be for all x-ray microscopy at high magnifications if confusion owing to overlying detail is to be avoided. To obtain the necessary image contrast with thin biological preparations, very soft x-rays must be employed.

The experiments described here were made with a projection x-ray microscope, as described by Nixon and Cosslett (1). Absorption in the several centimeters of air through which the rays pass in this apparatus must be eliminated if soft x-rays are to be used. This was done successfully by circulating helium through the enclosed specimen and photographic chamber. In our early trials, gold or nickel foils about 1 µ thick served as target windows for the x-ray tube operated at 3 to 6 kv. Under these conditions some image contrast was produced by thin specimens, but it was not adequate in photographs of sections of soft tissue. Far better results were obtained using



Fig. 1. (A) X-ray micrograph of a 10- $\mu$  section of frozen-dried mouse kidney. The paraffin-imbedded tissue was cut on a conventional rotary microtome. The section, mounted on a thin Formvar substrate, was deparaffinized before photography. (×180) (B) X-ray micrograph of decalcified human dentin. A 5- $\mu$  section of the methacrylate-imbedded tissue was cut on a rotary microtome equipped with a glass knife, was mounted in Formvar, and the plastic was removed. (×450).

aluminum as target window. Only a few percent of the K-radiation of aluminum, with a mean wavelength of 8 A, is absorbed in the helium, and very little white radiation of shorter wavelength is produced even when relatively high excitation voltages are applied to increase the efficiency of x-ray production. In this way very satisfactory photographs with exposure times of 5 to 10 minutes have been obtained by operating the tube at 10 to 15 kv, the target window being 7  $\mu$  of aluminum foil.

Figure 1 gives two examples of micrographs made under these conditions. The top picture (A) is of a 10- $\mu$  section through the cortex of frozen-dried mouse kidney at a magnification of about 180 times. The cells lining the convoluted tubules, which are the chief histological structures of this photograph, are discern-