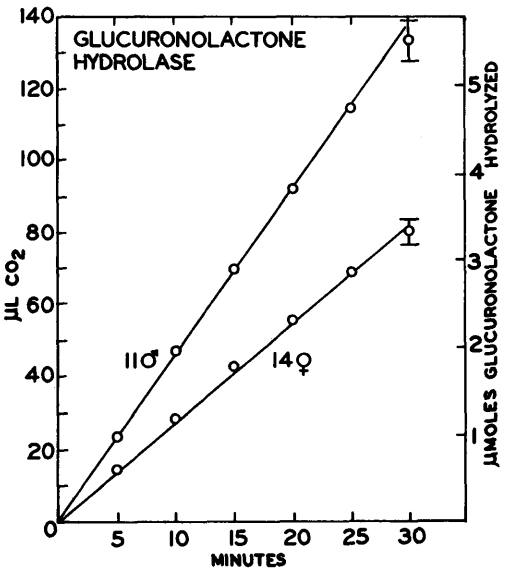

FIG. 4. Enzymatic activity of glucuronolactone hydrolase. Reaction rate is indicated by evolution of CO_2 (left ordinate) following enzymic hydrolysis of glucuronolactone to glucuronic acid in bicarbonate buffer. At 25°C 24.3 microliters is equivalent to hydrolysis of one micromole of lactone (right ordinate). Brackets around final points are standard error of mean.

latter is true, the differences in male and female tissue ascorbate would be direct consequences of the difference in blood ascorbate, which in turn could be the result of a sexual difference in hepatic biosynthesis. The hepatic enzyme differences reported here support this latter hypothesis. That is, the sexual differences in enzymic activities at the subcellular level could be the molecular basis for the differences in ascorbate concentrations found at the tissue level.

Summary. A sexual influence on the enzymes in the biosynthesis of ascorbic acid, and on the tissue concentrations of total ascorbate has been shown by comparisons between male and female rats. Males have significantly higher activities for the 4 hepatic enzymes assayed, and significantly higher ascorbate concentrations in 6 of the 8 tissues analyzed. The higher male enzymic activities at the subcellular level are thought to be the molecular basis for the higher concentrations of ascorbic acid at the tissue level.

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Radiosulfate Space in Humans at High Altitude.* (32349)

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It has long been known for many years that subjects acutely exposed to a high altitude environment lose weight(1,2), and it has been suggested(2) that this phenomenon may bear some relationship to the decrease in plasma volume which occurs under the same circumstances(2-6). However, we are not aware of any investigation concerning the extracellular space during acute exposure to high altitude. Picón-Reátegui(7) has reported a slight increase in the sucrose space in permanent residents of the high Andean region, in spite of the fact that their plasma volume is normal or moderately decreased(8). Data concerning the measurement of the intracellular space using radiosulfate are reported here. The observations have been carried out in sea level residents, before and

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TABLE I. Sulfate Space in High Altitude Natives and in Sea Level Residents Before and During Exposure to High Altitudes.

	Vol in liters	% of body wt
Sea level natives (14)	9.85 ± .33	15.02 ± .42
High altitude natives (7)	9.15 ± .52	16.41 ± .84
p values	>.20	>.10
Sea level natives before exposure to high altitude (9)	10.16 ±1.05	15.34 ± .53
Sea level natives 2nd day of exposure to high altitude (9)	8.79 ± .26	13.69 ± .49
p values	<.01	<.05

Numbers in parentheses are number of cases. Figures are means ± S.E.

during exposure to an altitude of 4,300 meters, and in men living constantly at this level.

Material and methods. We have studied, at sea level, 14 young adult male volunteers. Their age ranged from 19 to 27 years; their height from 162 to 177 cm. and their weight from 56 to 74 kg. Nine of them were restudied on the second day of exposure to high altitude. They were transported by train to Cerro de Pasco, Perú (4,300 m), where we also made similar observations in 7 high altitude natives, whose age ranged from 18 to 26 years, their height from 150 to 163 cm. and their weight from 53 to 63 kg. Each time 5 μC of $^{35}SO_4Na_2$ dissolved in 10 ml of saline were injected intravenously. Blood samples were drawn 10, 20 and 30 minutes afterwards. 0.2 ml of diluted plasma at 33% in water was transferred to a vial. To digest the proteins 0.3 ml of NCS (Nuclear Chicago) solubilizer was added(9) and after gently shaking, 3 ml of absolute ethanol and 10 ml of the fluorescent reagent(10). A vial with 0.2 ml of a standard solution was run simultaneously. Radioactivity was measured in a liquid scintillation system (Nuclear Chicago model 725). The radioactivity concentration was referred to percent of the dose. The volume of distribution was calculated using the extrapolation to 0 time, which was done by least squares.

Results. The results are summarized in Table I. High altitude natives have a sulfate space of 9.2 ± 0.5 liters, which is equivalent to 16.4 ± 0.8% of the body weight. Sea level natives have 9.9 ± 0.3 liters, 15.0 ± 0.4% of the body weight. The differences as liters or as percent of the body weight are not statistically significant ($P>0.20$ and $P>0.10$ respectively). In the group of sea level natives exposed to high altitude the results show a decrease in the sulfate space from 10.2 ± 1.1 to 8.8 ± 0.3 liters, the mean difference being 1.4 ± 0.2 with statistical significance ($P<0.01$). The body weight was also decreased from 66.3 ± 1.3 to 65.1 ± 1.2 kg. The mean of the individual differences was 1.2 ± 0.2 kg, statistically different from 0 with a $P<0.01$. The correlation coefficient between these 2 decreases was only 0.23 ± 0.14, and without significance ($P>0.10$).

Discussion. The results obtained show a reduction of the extracellular space during acute exposure to high altitude. The body weight was also decreased, but there was not a significant statistical correlation between the two phenomena. This may be due, at least in part, to the fact that the decrease in the extracellular space may occur simultaneously with an increase in the intracellular water of variable magnitude. It is known that acute hypoxia results in a reduction in plasma volume in the order of 400 ml(2,5), but the reduction observed in the extracellular space, which includes the plasma volume is about 3 times greater. Therefore, it seems reasonable to consider the plasma reduction as a consequence of the changes which occur in the extracellular space. The results obtained in high altitude natives show only small differences from the sea level controls, without statistical significance. Picon-Reátegui has reported a small increase of the extracellular fluid in high altitude natives(7). In either case, if we accept that newcomers tend to approach the conditions of high altitude natives, the suggestion can be made that the decrease observed in acute hypoxia must be of transient nature.

Summary. The extracellular space was studied in young adult males before and during acute exposure to an altitude of 4,300 meters, and also in a group of high altitude permanent residents. The results obtained indicate that during acute exposure to high altitude the sulfate space decreases 1.4 ± 0.2 liters ($P<0.01$) and that there is no dif-

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A Procedure for Explanting the 72-Hour Chick Embryo.* (32350)

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In an attempt to understand the mechanism of early mortality produced by ionizing radiations, we focused our studies on the circulatory system. The 72-hour chick embryo has a well developed circulatory system but *in ovo* is relatively inaccessible to microscopic study. Previously described techniques for explanting and culturing embryos for experimental manipulation and detailed microscopic studies were limited to embryos of less than 48 hours incubation(1-5). In the 72-hour embryo, the vascular area is too large and the embryo is too heavy to allow the required manipulation necessary for explantation without trauma to the embryo and the microcirculation. We have developed a technique for explanting the 72-hour embryo and the entire area vasculosa onto a solid agar-albumen medium. Such explanted embryos were cultured in a heated microscope chamber and detailed studies of the circulation in the transparent extraembryonic membrane were carried out. With this technique, we were able to maintain explanted embryos on an agar-albumen medium for periods up to 24 hours and in some cases as long as 30 hours.

Method and results. For our experiments, fertile eggs were incubated (Stage 18)(6) at a temperature of 38°C and 60% humidity. Equipment was sterilized and general aseptic procedures were employed.

The embryos were cultured on an agar-albumen medium that contained: 100 cc Howard-Ringer solution(7), 2 g agar, 1 g glucose, and about 50 cc thin albumen from unincubated eggs (about 6 eggs). The agar was added to the Howard-Ringer solution and the mixture was heated until the agar was completely in solution. The glucose was then added and the solution was cooled to about 50°C; the thin albumen was added and the solution was swirled gently until completely mixed. About 2 cc of this medium, enough to form a thin layer, was poured into small petri dishes (about 40 mm diameter) and the dishes were set aside to allow the medium to gel.

The shell and the shell membrane were cut off the blunt end of the egg, the albumen was removed, and the embryo and yolk were placed in a shallow finger bowl containing prewarmed Howard-Ringer solution (38°C). The yolk sac was cut at the equator and a modified spatula with a plastic ring (Fig. 1A) was inserted into the yolk (Fig. 1B). The ring was centered under the embryo, then the petri dish containing the medium was inverted over the embryo (Fig. 1C) and pressed down over the ring so that the vitelline membrane

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