changes did not reach their maximum until 72 hours after injection of papain.

The delay in response to injection of papain could be explained on the basis of the recently published data by Stockwell and Barnett(7), who showed that aging reduces the permeability of cartilage. On the basis of these data, it could be concluded that papain diffuses with diminishing ease through the cartilage matrix as the animals age and, therefore, produces not only progressively smaller chemical changes in the matrix, but also requires a longer time to achieve these changes.

The difference reported here between adult male and female rabbits to injection of papain cannot now be explained.

Summary. Following intravenous administration of papain to fully mature female rabbits, there is a decrease of borderline significance in the amount of extractable chondromucoprotein (CMP) in the cartilage matrix of the ear. In the adult male animals there is a statistically significant increase in the extractable CMP. Highly significant differences were also found in the amount of CMP between male and female animals 72 hours after injection of papain. The overall effectiveness of papain in causing chemical changes in cartilage matrix appears to decrease as the animals mature. Similarly, the maximal response to papain, which in baby rabbits occurs 24 hours after the injection and in adolescent animals at 48 hours, is delayed further until 72 hours post papain in the adult. The reason for this delay is believed to be decreased permeability of the cartilage matrix with aging.

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Effects of Prolonged Residence in the Antarctica upon Some Organic Constituents of Human Parotid Saliva. (29727)

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The chemistry of human whole mixed saliva(1-8) as well as parotid saliva(6,9-11) has been investigated in both oral(1,2,4,5,11) and systemic disease(3,6-11) as an index of change in physiological status.

As part of the International Geophysical Year, specimens of parotid saliva were obtained from a group of naval personnel(12) before and during their assignment to duty at McMurdo Sound, Antarctica. It was presumed that, in addition to a change in climate, prolonged residence under adverse living conditions including isolation, extended night, and extreme cold, might cause physiological changes reflected in saliva chemistry. As far as is known, similar data cannot be found in the existing literature. Additional data from this same population for blood, urine, and saliva will be reported by others (13).

Methods. Under paraffin stimulation, parotid saliva was collected from 29 enlisted men and officers at 5 different periods and analyzed for rate of flow, protein, tyrosine, tryptophan, and uric acid as previously described(11,14,15). One collection was obtained prior to departure for duty in Antarctica (July-August). Four collections were obtained in the Antarctica, *i.e.*, in the fall (February-March), winter (April-May), deep winter (June) and spring (September). The samples were quick-frozen, and shipped to

TABLE I. Effect of One Year's Residence in Antarctica upon Rate of Flow, Protein, Tyrosine, Tryptophan, and Uric Acid of Human Parotid Saliva.*

	Rate of flow,† ml/min	Protein, mg %	$\frac{\text{Pr(TCA)‡}}{\text{Pr(WPS)}} \times 100$	Tyrosine, mg %	Trypto-phan, mg %	Uric acid, mg %
			Control period			
Range	.2–2.4	23.2–218.5	27.3–72.7	.7–19.1	1.3–5.2	1.3–5.3
Mean ± S.E.	.7 ± .1	125.7 ± 8.5	43.7 ± 2.0	5.2 ± .8	2.8 ± .2	3.2 ± .2
			Experimental periods			
Fall						
Range	.3–1.4	38.0–308.9	9.3–83.0	1.5–16.7	1.9–8.3	1.5–5.2
Mean ± S.E.	.9 ± .1	160.1 ± 12.4	39.3 ± 3.4	6.7 ± .7	3.7 ± .3	3.2 ± .2
Winter						
Range	.3–2.2	22.4–232.8	21.6–78.1	1.2–14.3	1.2–5.8	1.7–6.0
Mean ± S.E.	.8 ± .1	157.2 ± 11.8	40.1 ± 2.4	6.3 ± .6	3.4 ± .6	3.6 ± .2
Deep winter						
Range	.3–2.2	54.2–282.0	14.7–52.3	2.3–11.3	1.9–8.3	1.4–6.8
Mean ± S.E.	.8 ± .1	147.4 ± 10.4	36.3 ± 2.8	5.8 ± .3	3.6 ± .3	3.9 ± .2
Spring						
Range	.2–2.0	19.9–312.0	20.4–57.3	.3–11.9	1.9–7.6	1.5–7.7
Mean ± S.E.	.8 ± .1	164.1 ± 13.4	37.7 ± 3.1	5.5 ± .6	3.9 ± .3	3.5 ± .3

* Based on 29 individuals.
† Includes saliva from both parotid glands.
‡ Pr, protein; TCA, trichlorcacetic acid; WPS, whole parotid saliva. Ratio represents that fraction of parotid salivary proteins precipitable by TCA.

continental U.S.A. by surface vessel. Saliva samples for analysis were thawed prior to use as needed and stored in an ice bath in a refrigerator at 4°C until completed.

Results and discussion. In the present longitudinal study, a controlled military population showed an increase in the protein, tyrosine, and tryptophan content in the parotid saliva under adverse living conditions in Antarctica.

Table I shows average values for rate of flow, protein, tyrosine, tryptophan, and uric acid over 5 periods of collection. As shown in Table II, the average protein values for the experimental periods were significantly higher by analysis of variance than those of the control period for the same individuals ($F<0.01$). The corresponding values for tyrosine and tryptophan were also significantly higher for the experimental than for the control period; namely, $P<0.05$ and $P<0.01$, respectively. Thus, the control parotid saliva contained 125 mg % protein, 5.2 mg % tyrosine and 2.8 mg % tryptophan. The experimental period averaged 157.2 mg % protein, 6.1 mg % tyrosine and 3.7 mg % tryptophan. No significant variations were seen between seasons in Antarctica.

The tyrosine and tryptophan concentrations of the parotid saliva varied concomitantly with the protein values during the experimental periods in Antarctica and thus confirmed previous findings(11). No differences were seen between control and experimental periods for rate of flow (0.8 ml/min), uric acid content (3.5 mg %) and for that portion of the salivary proteins precipitable by 30% trichloracetic acid.

TABLE II. Analysis of Variance for Rate of Flow, Protein, Tyrosine, Tryptophan, and Uric Acid After 1 Year's Residence in Antarctica.

	Rate of flow	Protein	$\frac{\text{Pr(TCA)}}{\text{Pr(WPS)}} \times 100$	Tyrosine	Tryptophan	Uric acid
Controls *vs* Antarctica	$F=3.3$ $P>.05$	$F=14.9$ $P<.01$	$F=.6$ $P>.05$	$F=4.3$ $P<.05$	$F=20.7$ $P<.01$	$F=2.0$ $P>.05$
Seasons within Antarctica	$F=1.0$ $P>.05$	$F=.9$ $P>.05$	$F=.8$ $P>.05$	$F=.3$ $P>.05$	$F=1.6$ $P>.05$	$F=1.6$ $P>.05$
Individuals	$F=4.6$ $P<.01$	$F=4.6$ $P<.01$	$F=4.1$ $P<.01$	$F=4.2$ $P<.01$	$F=4.8$ $P<.01$	$F=4.4$ $P<.01$

While differences between individuals (Table II) in the parameters examined were highly significant ($P<0.01$), a statistical evaluation of replicate controls indicates that the variation within individuals was significantly less than that between individuals. Thus, the longitudinal nature of this study in which each individual acted as his own control would be expected to minimize uncertain errors due to personal and genetic traits.

Many types of physiological insults have been reported to stimulate adrenocortical activity with associated changes in the chemistry of blood and urine(16). For example, the concentration and distribution of blood and urinary proteins have been shown to be affected by cold exposure(17-20). In man, a proteinuria has been observed in association with cold pressor tests(17,18), and cold exposed rats and rabbits showed a changed electrophoretic distribution of the plasma proteins(19,20). It should be emphasized that, in the present study, factors other than exposures to cold may have influenced the protein content of the parotid saliva.

It is of interest that recently Weber has indicated that individuals under reduced pressure in manned space cabin simulators showed a decreased concentration of total protein in the parotid saliva(21). The conditions predisposing to changes in salivary protein content, however, are quite different than those in our study.

Summary. 1. The parotid salivas of 29 naval personnel have been examined for rate of flow, protein, tyrosine, tryptophan and uric acid before and during assignment to duty at McMurdo Sound, Antarctica. 2. The control parotid salivas contained 125 mg % protein, 5.2 mg % tyrosine, 2.8 mg % tryptophan. On a grouped basis, the saliva during the experimental period contained 157.2 mg % protein, 6.1 mg % tyrosine, and 3.7 mg % tryptophan ($P<0.01$, <0.05, <0.01, respectively). 3. No differences were observed between control and experimental periods for rate of flow, uric acid content, or for that portion of salivary protein precipitable with 30% TCA. The mean values were 0.8 ml/min, 3.5 mg % and 38.4%, respectively. 4. Differences between individuals were highly significant. No significant variations were observed between seasons in Antarctica.

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Lactic Dehydrogenase Isozymes During Development of Azo Dye Tumors. (29728)

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According to current thinking the various molecular forms of lactic dehydrogenase (LDH) in most animal tissues are the result of random tetrameric associations of 2 sub-units, the synthesis of each sub-unit being under the control of a separate gene(1,2,3). In tissues one may expect to find up to 5 distinct forms (isozymes) of LDH(2,4). The relative distribution of the isozymes is tissue specific, and in various diseases changes in serum LDH reflect the isozymic pattern of the affected tissue(5,6,7). That the isozyme pattern is not "static" throughout the life of an organism is indicated by the fact that the distribution in fetal tissue is usually different from that of the adult tissue(4,8,9). In this connection Kaplan and his co-workers have advanced the thesis that the various isozymes, although having the same catalytic specificities, possess different physiological functions(8,10). Several lines of evidence have led these workers to postulate that LDH-1 (the isozyme with the greatest mobility toward the anode) more readily allows for the further aerobic oxidation of pyruvate than does LDH-5 (the isozyme with the least mobility toward the anode). On the other hand, LDH-5 seems to be more involved in anaerobic processes by more effectively catalyzing pyruvate reduction. Developmental changes as well as the observed tissue specificity of LDH isozymes tend to support this thesis.

In view of the above findings it was of interest to ascertain whether changes occurred in the distribution of LDH isozymes during the induction of liver tumors in the rat. For this purpose, the hepatocarcinogen 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) was employed. This compound, at the concentration fed, must be administered for a considerable period (about 60 days) before evidence of tumor appears (11,12,13,14).

Methods. Young adult male rats (Holtzman), weighing approximately 300 g, were fed the semi-synthetic diet employed by Clayton and Abbott(15) to which 0.064% 3′-Me-DAB was added. Control animals were fed the same diet without the dye. Both food and water were given *ad lib.* At stated time intervals animals were killed by decapitation and tissues were removed and rinsed. They were then cut into small pieces, thoroughly washed, homogenized, and centrifuged. The supernatants were used for the reported studies.

LDH was assayed by measuring the rate of NADH oxidation at 340 mμ in a Zeiss PMQII spectrophotometer. The reaction mixture of 3.0 ml contained the following: 200 μmoles of potassium phosphate buffer, pH 7.4, 0.26 μmoles of NADH, 0.96 μmoles of sodium pyruvate, and the tissue extract. Measurements were carried out at 30°.

Vertical starch gel electrophoresis was based on the procedure of Smithies(16). The gels were buffered at pH 8.6 according to the procedure of Boyer, Fainer and Naughton(17), and the resulting zymogram developed with the reaction mixture employed by Markert and Ursprung(18). For each electrophoresis experiment all extracts were diluted to contain the same total LDH activity and were developed under the same conditions.

Results and discussion. Table I shows the LDH activity in rat liver during the time course of tumor induction by 3′-Me-DAB.