activity of the organism. He has sketched the organization of the neural processes involved, and has proceeded with patience, ingenuity, and steady attention to openings for further test. In an address to architects a few years ago (*1*) he summed up: "While in no way denying the existence of the 'external world' our disclosures apparently show that the only aspects of it man can know anything about are those aspects which are either helpful or thwarting in carrying out his purposes."

In harmony with Ames' work is that of Hoyt Sherman at Ohio State University in which unexpected abilities have been aroused in students by a drawing technique that organizes the total visual field with the muscular requirements of the procedure under way (*34*).

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Technical Papers

Prolongation of the Fertilizing Capacity of Sea-Urchin Spermatozoa by Amino Acids

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The life span of sea-urchin spermatozoa can be prolonged by addition of various agents to the sea water in which they are suspended (*1*). Hayashi (*2*) found that dilution of the sperm with seminal fluid instead of sea water extends the fertilizing capacity considerably and that the effective agent is most probably a protein. Chang (*3*) has obtained a similar effect of seminal fluid on spermatozoa of rabbits. Metz (*4*) noted that addition of hen's egg white increases the fertilizing power of starfish sperm. Wicklund (*5*) found that bovine serum albumin, trypsin, and chymotrypsin maintain very well the fertilizing power of dilute suspensions of sea-urchin spermatozoa, whereas glucose and fructose are somewhat less effective.

We have confirmed the effect of these proteins on spermatozoa of the sea urchins *Lytechinus pictus* and *Strongylocentrotus purpuratus*. In addition, we find that various amino acids give more marked extension of the functional life of the spermatozoa. The amino acids thus far tested include glycine, alanine, valine, leucine, and lysine, and all are found to be active in this regard. The peptide glutathione was also tested and found to be effective.

The tests were made with both relatively dilute (*ca.* 0.5%) and relatively concentrated (*ca.* 5%) sperm sus-

TABLE 1

EFFECT OF GLYCINE ON THE DURATION OF FERTILIZING CAPACITY OF SPERMATOZOA OF THE SEA URCHIN *Lytechinus pictus*

Sperm concentration	Age of suspension	ml of sperm used for insemination	Percentage fertilization with sperm aged in	
			0.05 *M* glycine in sea water	Sea water
0.4%	10 min	0.05	0.2	0
	"	.2	8	2
	"	.8	95	8
	20 min	.05	0	0
	"	.2	10	0
	"	.8	65	0
	4 hr	.2	10	0
	"	.8	100	0
	7 1/2 hr	.2	10	0
	"	0.8	40	0
	24 hr	2.0	25	0
5%	4 1/2 hr	0.05	100	20
	"	.1	100	70
	"	.2	95	100
	5 5/6 hr	.05	100	0.5
	"	.1	100	15
	"	.2	100	40
	6 3/4 hr	.05	100	0
	"	.1	100	0
	"	.2	100	0
	23 hr	.05	0	0
	"	.1	10	0
	"	0.2	90	0

pensions. As is well known (*6–9*), the life span of the spermatozoa in sea water decreases with increasing dilution of the suspension. The maintenance of fertilizing capacity by addition of amino acids was obtained with both concentrated and dilute suspensions, the latter exhibiting the more marked relative prolongation. The suspensions were allowed to age at room temperature (20°–22° C) in flasks on a slow shaker, or unshaken in a shallow layer in Petri dishes. The solutions were made up in sea water and adjusted to the pH of sea water. Inseminations were made with various amounts of control and treated sperm added to 5 ml of sea water containing about 400 eggs, and percentages of fertilization were determined from both membrane elevation and cleavage. In Table 1 a sample of the results obtained with glycine is presented.

The data of 12 sets of experiments with 0.05 *M* glycine consistently show a prolongation of fertilizing capacity which, for dilute suspensions, amounts to more than fifty times that of the controls. Tests with 0.01 and 0.1 *M* solutions gave less extensive prolongation than did the 0.05 *M* solutions. When glycine is added to sea water-senescent spermatozoa, there is an improvement in their fertilizing capacity, but this does not attain the value exhibited by the spermatozoa that have been in glycine from the start.

Runnström *et al.* (*10*) and Wicklund and Gustafson (*11*) found that when underripe sea-urchin eggs, which fail to fertilize or to form good membranes, are pretreated with, and inseminated in the presence of, glycine good fertilization and membrane elevation are obtained. We have confirmed this interesting discovery and find, in addition, that the effect is obtained by treatment of the sperm alone. There is, of course, a small amount of glycine carried over with the sperm, but control tests show that the presence of such small amounts during fertilization has very little, if any, effect. Also, pretreatment of the eggs with glycine gave no such improvement in fertilization or membrane elevation as is obtained by treating only the sperm and inseminating in sea water.

Sea-urchin spermatozoa that have been reversibly agglutinated by fertilizin suffer an impairment of fertilizing capacity (*12*). Treatment with glycine improves somewhat the fertilizing power of such suspensions, but it remains far below that of the controls. The results are interpretable as an effect of the glycine on a fraction of spermatozoa that had not reacted with the fertilizin. Tests were also made of the effect of glycine on the agglutination of the sperm by fertilizin. The titer of agglutination was found to be unaffected. However, the spontaneous reversal of agglutination occurred considerably (roughly three times) more rapidly. This is consistent with the increased activity that the spermatozoa exhibit in the glycine solutions, since the rate and degree of spontaneous reversal correlate with degree of activity (*12*). Determinations were also made of the liberation of antifertilizin from the sperm upon aging. This was found to occur earlier and in higher titer in sea water than in the glycine solution. The presence of the amino acid evidently opposes the dissolution of this surface constituent of the spermatozoon.

In this connection Metz (*13*) has recently found that various α-amino acids, including the ones used in these experiments, act as adjuvants for the agglutination of starfish sperm by fertilizin. It appears, then, that the action of the amino acids in maintaining fertilizing capacity may involve an effect on the antifertilizin of the sperm.

Since the spermatozoa in glycine are maintained in a state of high activity it is evident that glycine also has a metabolic effect. One possibility is that the amino acid is oxidized. However, determinations of ammonia (which is one of the products of oxidation) showed no significant production. Also tests for utilization of glycine by the sperm, by the method of Alexander *et al.* (*14*), showed no appreciable disappearance. Ammonia was also tested (at the pH of sea water) for possible action in prolonging the life span of the sperms and was found to be effective, although much less so than glycine. At 0.05 *M* it proved deleterious, but concentrations from 0.01 to 0.0005 *M* were found to be increasingly favorable. It is possible, then, that the effective agent in the action of the amino acids may be ammonia continuously produced in amounts too small to be readily detected chemically.

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Polarographic Measurement of the Oxygen Consumption of Skin *in Vivo*[1]

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Manometric measurements have shown that oxygen consumption of the skin is markedly changed by inflammatory diseases, neoplasms, hormonal influences, age, irradiation, and vesicants (*1–4*). The present study utilizes a polarographic method for estimating oxygen tension and oxygen consumption of human skin (*5, 6*). The results in normal skin are presented.

Polarographic measurement of cutaneous oxygen consumption is based on the observation that removal of oxygen supply by blanching the skin results in a rapid fall of the oxygen tension to very low levels in 1½–2 min. This is achieved by elevating the tip of an electrode, inserted intracutaneously, until enough pressure is applied to the overlying skin to force the blood out of a 5–6-mm² area (*6*). An estimate of the relative rates of the oxygen consumption of tissues can be obtained from the rate of fall of the oxygen tension.

One hundred and thirty experiments were performed within a 20-cm² area of the extensor surface of the left forearm of 3 healthy white male students. The electrodes were inserted into the skin to depths corresponding approximately to those of the epidermis and corium and into hair follicles. Control experiments were carried out on excised human skin stored for 4 days, and on the abdominal skin of an anesthetized dog after intradermal injection of 0.1 ml of 0.1 *M* sodium azide. Galvanometer readings were recorded every 15 sec throughout the experiment, following stabilization of the electrolysis current.

Elevation of the electrode tip results in a movement artifact, which consists of a partial fall of the electrolysis current. The first reading after the application of pressure was not considered in the evaluation of the experimental results because of the movement artifact.

The fall in oxygen tension in human skin, plotted against time on semilog paper, was found to approach a straight line. The oxygen consumption of normal human epidermis was significantly greater than that of normal corium in the same area. Extremely rapid oxygen uptake was found in about 30% of the experiments in which electrodes had been inserted into hair follicles. It seems possible that in these cases the tip of the electrode entered a sebaceous gland (Fig. 1).

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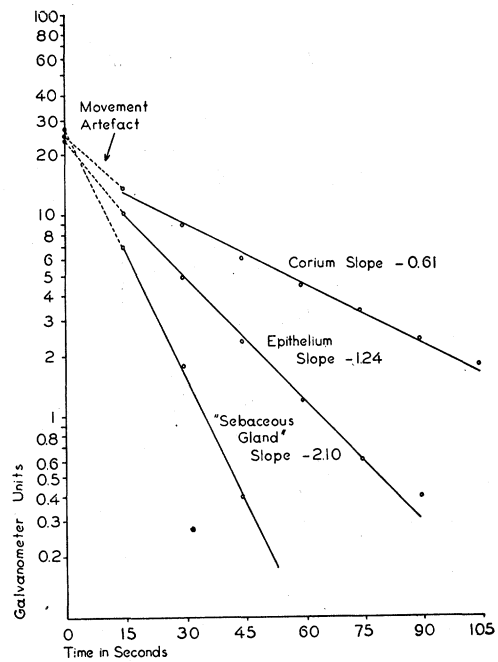

FIG. 1. Relative oxygen uptake of corium, epithelium, and "sebaceous glands" of normal human skin measured polarographically. Oxygen tension is expressed in galvanometer units.

The frequency distribution curve of the slopes obtained from the experiments on normal human skin shows 3 maxima. These correspond to the 3 levels of the skin to which the electrodes were inserted, and are related to one another in the same way as the QO_2 values reported for connective tissue, skin, and glandular tissue in manometric studies (Table 1).

Immediate repetition of the measurements in the same skin area without reinsertion of the electrode significantly decreased the oxygen consumption. This was probably a result of injury to the skin.

TABLE 1

RELATIONSHIP OF RELATIVE OXYGEN UPTAKE OF CORIUM, EPIDERMIS, AND "SEBACEOUS GLANDS" MEASURED POLAROGRAPHICALLY TO QO_2 OF HISTOLOGICALLY SIMILAR TISSUE OBTAINED BY MANOMETRIC METHODS (*1, 7*)

Region of skin	Logarithm of mean slope	$\frac{10}{\text{Slope}}$	Tissue	QO_2
Corium	−0.65	0.44	Connective tissue	0.4
Epidermis	−1.30	2.00	Skin (*1*)	2.1
"Sebaceous gland"	−2.00	10.00	Liver (7)	9.0