with silicone or a nontoxic plastic cement.

The design of this dish should aid the investigator in obtaining better cytological detail of monolayer cultures without increasing the danger of contamination and should be especially useful in laboratories where frequent screening is required of large numbers of plates as well as in conducting student laboratory experiments where the supply of inverted microscopes is inadequate.

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Effect of Glutamate and Glycine on Cock Sperm Metabolism. (26481)

S. EL ZAYAT AND A. VAN TIENHOVEN (Introduced by M. L. Scott)
Agricultural Experiment Station, Cornell University, Ithaca, N. Y.

During investigations on the effect of chloride on cock sperm metabolism it was found that replacement of chloride by glutamate in a Tyrode diluent resulted in a linear depression of the rate of fructolysis and of respiration rate(1). Replacement of chloride by phosphate did not result in a significant change in rate of metabolism. The present paper reports results obtained in a further investigation of these phenomena.

Materials and methods. The methods used to obtain semen, to measure respiration rate and utilization of fructolysis have been previously described(1). In all experiments a 1:10 dilution rate was used. All experiments were designed as completely randomized blocks and analyzed according to the methods outlined by Snedecor(2). Diluents were made isotonic to seminal plasma ($\Delta = 0.62°C$). Freezing points were determined with the aid of a Fiske milkcryoscope. Compositions of the basic diluents employed are given in Table I. A designation of 25% "phosphate" means that 3 parts of "glutamate" diluent were mixed with 1 part of "complete phosphate" diluent. All diluents contained 100 mg dihydro streptomyocin and 10 mg tetracycline hydrochloride per 100 ml(3). To determine the catabolism of glutamate 6 μc of glutamate 1-C^{14} were added per Warburg flask; a similar procedure was used in the case of glycine. The $C^{14}O_2$ was trapped in KOH and either converted to $BaC^{14}O_3$ and the radioactivity of $BaC^{14}O_3$ determined, or the KOH and $KHC^{14}O_3$ were plated directly on in a small metal cup and radioactivity measured on the dried material. Radioactivity was measured with a thin mica window Geiger-Müller counter.

Experiments and results. The effect of replacement of chloride by phosphate or glutamate was investigated in an experiment in which the 3 diluents were used simultaneously. The results are presented in Table II. Analysis of variance revealed: 1) Respiration rates of Tyrode-diluted and phosphate-diluted fowl semen are higher ($P<0.001$) than that of glutamate-diluted semen; 2) Diluents had no significant ($P>0.20$) effect on rate of fructolysis. 3) The interaction be-

TABLE I. Composition of Diluents Used in Fowl Semen Experiments.

Isotonic solution	g/100 ml	"Complete phosphate"	Tyrode	"Glutamate"
		ml	ml	ml
$CaCl_2 \cdot 2H_2O$	2.04	1.00	1.00	1.00
$MgCl_2 \cdot 6H_2O$	2.48	.40	.40	.40
KCl	1.25	1.60	1.60	1.60
Na H CO_3	1.53	6.50	6.50	6.50
Fructose	6.00	4.00	4.00	4.00
Na monoglutamate	3.21	—	—	86.50
Phosphate buffer*	—	86.50	—	—
Na Cl	.97	—	86.50	—

* 16.34 g Na_2HPO_4 plus 5.16 g NaH_2PO_4 in 1000 cc water.

TABLE II. Effect of Phosphate and Glutamate Replacement of Chloride in Tyrode Diluent on Metabolic Rates of Fowl Semen.

Diluent	Respiration rate $\mu l O_2/10^8/1$ hr	Respiration rate $\mu l O_2/10^8/2$ hr	Fructose used, $\mu g/10^8/2$ hr
Tyrode	4.11	6.95	62.47
Complete phosphate	4.22	6.11	13.73
Glutamate	3.39	5.37	17.76
N	12	12	6

tween duration of incubation and diluents was significant ($P<0.05$). The interactions (Tyrode versus Phosphate + Glutamate) $\times$ duration of incubation were significant ($P<0.05$) but the comparison (Phosphate *vs.* Glutamate) $\times$ duration of incubation was not ($P>0.20$). These interactions suggested that phosphate might have no effect on respiration rate during the first hour of incubation but that it might depress respiration later in the incubation period. Such an effect would be similar to that obtained by addition of glycine to a Tyrode diluent(4).

To investigate this problem further we incubated semen diluted with the 3 diluents, mentioned above, for 6 hours. Hourly respiration rates observed in these experiments are given in Table III together with correlation and regression coefficients of hourly respiration rate on time. To make comparisons with previously published data on glycine we recalculated the coefficients for the first 6 hour period in those experiments. These data show: 1) A remarkable agreement in regression coefficients obtained with Tyrode diluent in 2 different experiments. 2) Variations in hourly respiration rate of semen diluted with glutamate and phosphate diluent showed no significant relationship with duration of incubation. We may conclude from this that either there was no relationship and thus the variations were random, indicating that the lines were horizontal, or that the regressions although real were not significant because not enough points were available to demonstrate this relationship. In either case we may conclude that the regression lines for Tyrode-diluted semen and those for phosphate-diluted and glutamate-diluted semen will cross. This in turn means that phosphate or glutamate prevents the gradual decrease in respiration rate observed with Tyrode-diluted semen.

TABLE III. Hourly Respiration Rates for Fowl Semen Diluted with 3 Different Diluents and Calculated Regression (b) and Correlation Coefficients (r) of These Respiration Rates on Time of Incubation.

Hr	Tyrode	Complete phosphate	Glutamate
	$\mu l O_2/10^8/hr$		
1	3.85	3.99	2.90
2	2.46	1.49	1.60
3	2.77	2.49	2.27
4	1.75	1.59	1.53
5	1.96	2.06	2.31
6	1.90	2.19	1.42

Diluent	N	b	P	r	P
Tyrode	6	.3508	<.05	.735	>.05
Complete phosphate	6	.2337	>.10	.482	>.05
Glutamate	6	.1714	>.10	.552	>.05
Tyrode*	3	.3514	<.05	.837	>.05
Tyrode - glycine*	3	.1420	<.01	.925	<.01

* See text.

These experiments suggested 2 further questions: 1) what is the effect of graded replacement of phosphate by glutamate, in the complete phosphate diluent, on metabolic rates? 2) Are glutamate and/or glycine catabolized by the fowl semen?

Results of investigations of the first question are given in Table IV. Analysis of variance showed that: 1) The quadratic components of the effect of phosphate replacement by glutamate were significant ($P<0.01$). 2) Differences in amounts of fructose used were not significant ($P>0.10$).

These data thus indicated that there was an optimum ratio of phosphate and glutamate with respect to respiration rate, and that this optimum lies near the 50:50 ratio. The significance of the apparent discrepancies between data of Table II and Table IV is discussed below.

Data concerning the second question raised above are summarized in Table V. These results demonstrate: 1) glutamate depresses rate of fructolysis under aerobic and anaerobic conditions; 2) glutamate depresses respiration rate; 3) glycine depresses respiration rate; 4) glutamate is catabolized to a limited extent by fowl semen; 5) rate of catabolism of glycine by fowl semen is negligible; 6) analysis of variance showed that the dif-

TABLE IV. Effect of Graded Levels of Glutamate and Phosphate on Metabolic Rates of Fowl Semen.

Diluent	Respiration rate $\mu l O_2/10^8/1$ hr	$\mu l O_2/10^8/2$ hr	Fructose used, $\mu g/10^8/2$ hr
Phosphate			
100%	3.06	5.21	24.47
75%	3.13	5.25	29.39
50%	3.39	5.48	25.16
25%	3.26	5.49	26.13
Glutamate			
100%	2.81	5.15	23.10
N	7	7	4

ference in fructose used under aerobic and anaerobic conditions was not significant ($P>0.05$).

These results show that glutamate, in contrast with glycine, can be catabolized by fowl semen. With respect to glycine fowl sperm resemble sea-urchin sperm(5) but differ from bovine sperm(6,7). We tried to approximate whether the catabolism of glutamic acid might account for the smaller amount of fructose used under aerobic conditions. In these calculations we assumed that all fructose which disappeared had been metabolized to lactic acid(4) yielding 2 moles ATP for each mole of fructose used and we assumed that 36 moles of ATP would be generated per mole of glutamate catabolized to CO_2. Based on these assumptions we made the following calculations: ATP generated in Tyrode diluent from fructolysis 2.678 micro moles; ATP generated in glutamate diluent: 0.165 micro moles from fructolysis plus 1.494 micro moles from glutamate thus yielding 1.65 g micro moles. Thus approximately 1 micro mole of ATP less is generated in the glutamate diluent. This calculation does not take account of differences in respiration rates in the 2 diluents since those differences may be due to chelating effects of glutamate. The data obtained under anaerobic conditions show clearly that glutamate has an inhibitory effect on fructolysis even when glutamate cannot be catabolized. Under these conditions about 0.7 micro moles less of ATP are generated in the glutamate than in the Tyrode diluent.

Discussion. The results showing that chloride replacement by phosphate in a Tyrode diluent had no significant effect on respiratory or fructolytic rate of fowl sperm agree with previously obtained results(1). The inhibitory effect of glutamate on respiration also agrees with these results. Comparisons of the means obtained given in Tables II and V, and in Table IV, show that the magnitude of the difference in respiration rate between phosphate-diluted and glutamate-diluted semen varied. We would almost certainly not have ascribed an inhibitory effect of glutamate on respiration on the basis of data given in Table IV. These data do, however, not vitiate the conclusions drawn from Tables II and V. They indicate that the extent of the response may vary.

What factors are involved in this variation we do not know for to the best of our knowledge identical laboratory methods were used. We have no evidence that ageing of the roosters or that season of collection of the semen affects the response. It must be emphasized that our conclusions are based on incubation periods of 2-3 hours. As hourly respiration rate decreases more sharply after dilution with Tyrode diluent than with the other diluents, this restriction must be kept in mind in comparisons of diluents.

We believe that the conclusion that a higher respiration rate is obtained with a 50:

TABLE V. Effect of Glutamate and Glycine on Metabolic Rates of Fowl Semen.

Diluent	Respiration, $\mu l/10^8/3$ hr	Fructose used, $\mu g/10^8/3$ hr Aerobic	Anaerobic	Amino acid used, $\mu g/10^8/3$ hr	N
Tyrode	11.71	241.22	161.70	—	4
Glutamate	6.25*	14.88*	30.92*	6.1	4
Tyrode	13.14	—	—	—	9
Tyrode (84p) + 1/3 M glycine (16p)	7.87*	—	—	.66	9

* Different from treatment without amino acid ($P<.01$).

50 ratio of phosphate buffer and glutamate is not jeopardized by the somewhat lower than "normal" rate of respiration obtained with 100% phosphate, since experimental results are based on randomized complete block designs and the higher respiration rate was observed in 5 out of 7 cases. The nature of this synergestic effect of glutamate and phosphate is not clear, mainly because so little is known of the metabolism of fowl sperm.

The sharper decrease in respiration rate after dilution of fowl semen with Tyrode diluent may be related to the distinctive action of the chloride ions on sperm(1). Koefoed-Johnsen and Mann(9), observed that surfactants caused inhibition of fructolysis, respiration and motility. The chloride ion probably does not cause immediate disruption of the normal sperm morphology but eventually may act as surfactants do. Whether sperm stored in Tyrode diluent respond with a higher respiration rate to succinate than do phosphate diluted stored sperm should be investigated. Such a phenomenon was observed by Koefoed-Johnsen and Mann(9) after treatment of mammalian semen with surfactants.

The inhibition of respiratory activity by glutamate is, however, not attributable to replacement of chloride, for glutamate also inhibited respiration rate when compared to a phosphate diluent. Other data from our laboratory did not give evidence that phosphate causes damage to fowl sperm(1).

Glycine, which is known to act as a chelating agent(9), and glutamate may have a similar effect on respiratory activity partly because of the shared characteristic of acting as a chelating agent. In view of the fact that tetracycline hydrochloride was added to all diluents and in view of its chelating effect (10) it seems that further investigation is necessary.

Summary. Glutamate, which can be catabolized by fowl semen, has an inhibitory effect on respiratory rate and in some experiments on rate of fructolysis. This effect may be explained partly by the catabolism of the glutamate and partly by its chelating effect. Glycine, which acts similarly to glutamate, is not catabolized by fowl semen.

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Effect of Hypophysectomy on Adrenocortical Response to Bilateral Carotid Constriction. (26482)

EDWARD G. BIGLIERI AND WILLIAM F. GANONG
Metabolic Unit for Research in Arthritis and Allied Diseases and Department of Physiology, University of California School of Medicine, San Francisco

Gann, Mills and Bartter(1) have demonstrated that bilateral constriction of the common carotid artery causes an increase in aldosterone secretion in normal dogs. Considerable evidence has accumulated that ACTH affects aldosterone secretion(2,3). This fact makes it difficult to decide whether increased aldosterone secretion in any given experiment is due to increased secretion of ACTH or to a proposed nonpituitary aldos-