ance of avoiding surgical trauma to the blood supply of the stomach.

This failure of vagal stimulation to release gastrin is consistent with the large body of experimental evidence which indicates that the mechanisms of the cephalic (neural) phase and of the gastric (hormonal) phase of acid secretion may operate quite independently of each other. This does not deny the possibility of additive or synergistic action between these two mechanisms. Nor does the present report controvert the evidence which indicated that local nervous pathways are involved in the release of gastrin by food in the stomach or by antral distention, but these are purely local(8).

Conclusion. The failure of vagally denervated canine gastric pouches to secrete acid in response to intense vagal stimulation of the main stomach constitutes critical evidence that vagal stimulation does not release gastrin.

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Metabolism of Sea Urchin Spermatozoa and Induced Anaerobic Motility in Solutions of Amino Acids. (18386)

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The addition of any one of a number of different amino acids and peptides to suspensions of sea urchin spermatozoa in sea water has been found greatly to extend their functional life span(1). The period during which the spermatozoa retain their fertilizing capacity in dilute suspensions, can be increased more than 50-fold by means of these agents. During the period of prolonged life span the spermatozoa remain highly motile.

In contrast with mammalian spermatozoa, the metabolism of sea urchin spermatozoa is predominantly aerobic(2). In the absence of oxygen the spermatozoa soon become motionless(3,4). Ordinarily, the respiration of sea urchin spermatozoa decreases with time following dilution, from a high initial rate to practically zero, concomitant with loss of motility. It might, then, be expected that during the period of extended life and continuing motility induced by added amino acid or peptide the respiration would remain at a level similar to that of a freshly diluted suspension in ordinary sea water. The results of the present experiments show that such is not the case.

Material and methods. The sea urchins *Arbacia punctulata* (obtained at Woods Hole, Mass.) and *Lytechinus pictus* (obtained at Corona del Mar, Calif.) were used in this work. The semen was obtained by the KCl-injection method(5). Oxygen uptake was measured by means of Barcroft-Warburg manometers with conical, single-side arm vessels of 16 to 20 ml volume in which sperm suspensions of 3 ml final volume were generally used. Shaking was 85 to 95 c.p.m. and 3 to 4 cm stroke. The amino acid solutions were made isosmotic with sea water, at the pH of sea water, and diluted with sea water to the desired concentration. Since, in prolonged manometric experiments, growth of bacteria may be a factor in the results, determinations of their presence were made by microscopic examination and, in certain experiments, by platings on medium suitable for the growth of marine bacteria(6). In addition use was made of penicillin in many of the experiments, to check bacterial growth.

* Part of these investigations were carried out at the Marine Biological Laboratory, Woods Hole, Mass., with the assistance of Mr. Richard Glass.

1. Tyler, A., *Biol. Bull.*, 1950, v99, 324.
2. Rothschild, Lord, *Biol. Rev.*, 1951, v26, 1.
3. Harvey, E. B., *Biol. Bull.*, 1930, v58, 288.
4. Barron, E. S. G., *Biol. Bull.*, 1932, v62, 46.
5. Tyler, A., *The Collecting Net*, 1949, v19, 19.
6. Waksman, S. A., Renszer, H. W., Carey, C. L., Hotchkiss, M., and Renn, C. E., *Biol. Bull.*, 1933, v64, 183.

Results. Use of penicillin. In dilute suspensions of spermatozoa the rate of oxygen uptake decreases with time to barely measureable values following which there occurs a rapid increase in rate. In concentrated suspensions the secondary increase may occur before the initial decline in rate has reached zero. While it has been suggested that the secondary rise might be due to autolysis, it seemed more likely that bacteria, which are visibly present in increasingly large numbers in aging sperm suspensions would be responsible for the phenomenon. In order to test this, as well as to provide a method for following the respiration of sperm suspensions for prolonged periods, the action of penicillin (Penicillin G. Potassium; Squibb) was examined. The tests showed that this agent, at a concentration of 50 units per ml, was effective in checking the growth of the marine bacteria and in preventing the secondary rise in respiratory rate of the aging sperm suspension.

Fig. 1 illustrates the results of one such experiment in which oxygen uptake was followed for 17 hours. While the rate of respiration of the sperm in ordinary sea water is seen to rise rapidly from 12 hours on, that in the penicillin-sea water remains at its low level. Bacterial counts, made from agar-plates of serial dilutions of the contents of the vessels at the end of the experiment (17 hours), showed an average of 1.7×10^8 per ml for the sperm suspension in sea water and 1.8×10^2 per ml for the suspension in penicillin-sea water. The latter value is of the same order of magnitude as the bacterial counts of the original sea water (5×10^2) and the penicillin-sea water (6×10^2). Similar low bacterial counts were obtained in eleven other sets of platings from 20-hour sperm suspensions in penicillin (50 units per ml) sea water. The penicillin does not, however, indefinitely suppress bacterial growth, and the concomitant rise in respiratory rate. This is presumably due to the appearance of penicillin-resistant mutants, or the growth of penicillin-resistant types originally present, or deterioration of the penicillin in solution. From the present results, it is clear that the addition of penicillin is of considerable utility in enabling prolonged measurements to be made on the respiration of aging sperm suspensions. It should, also, be noted that penicillin, at the concentration employed (50 units per ml), does not affect the respiration of the spermatozoa.

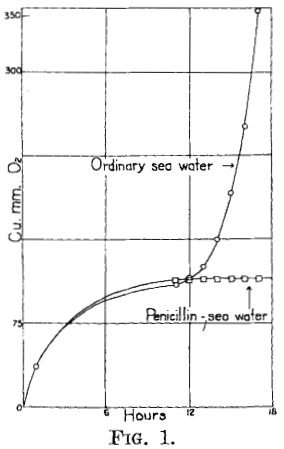

FIG. 1.

Oxygen uptake of senescing spermatozoa of *Lytechinus pictus* in ordinary sea water and in sea water containing 50 units of penicillin per ml. Suspension: approximately 2.5×10^8 spermatozoa in 3 ml. Temp., 22°C. See text for further data.

Action of amino acids. The respiration of aging sperm suspensions was examined in 25 sets of manometric experiments employing duplicate, triplicate, or quadruplicate vessels for the amino acid-treated and the control sperm. The amino acids tested were glycine, alanine and histidine. Tests of fertilizing capacity and examinations of motility were also made on the sperm suspensions from the respiration vessels. Since the results of the experiments were essentially similar, it will suffice to describe typical sets.

Fig. 2 illustrates the results of an experiment consisting of 3 control vessels of sperm in ordinary sea water and 3 vessels with the sperm in 0.05 molar glycine. The mixing of the sperm with the glycine solution, and with the corresponding volume of sea water, took place just after the first reading of the manometers by tipping the sperm from the side arm of the vessels. The readings began ½ hour after initial dilution of the sperm to 3 per cent suspension. The final concentration in the vessels was one per cent and the sperm count of the latter was 2×10^8 per ml. The

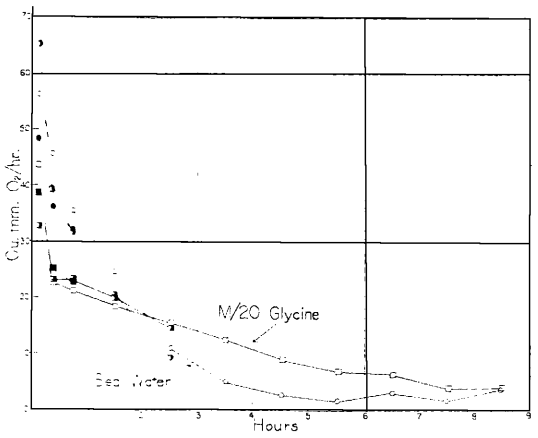

FIG. 2.

Rates of oxygen uptake of senescing suspensions of spermatozoa of *Lytechinus pictus* in sea water (circles) and in M/20 glycine in sea water (squares). Suspension: approximately 6 × 10^8 spermatozoa in 3 ml. Glycine or sea water (2 ml) mixed with sperm suspension (1 ml in side-arm) immediately after initial reading of manometers. Original dilution of semen at ½ hr before first reading. Temp., 22°C.

following features of the results may be noted. (a) Both glycine-treated and control sperm show a high initial rate of oxygen uptake which drops rapidly to relatively low values. (b) The initial rate of the sperm in glycine is markedly lower than that of the controls. (c) The rate declines less rapidly in glycine so that after the second hour the treated sperm respire at a somewhat higher rate than the controls.

In this experiment pairs of vessels were removed at 1, 3 and 9 hours at which time the sperm were tested for fertilizing capacity and examined for degree of motility. Fertilizing capacity was determined by inseminating aliquots of eggs with serially diluted amounts of sperm taken from the vessels and can be expressed, in terms of the reciprocals of the relative amounts of treated and control sperm required to give a definite percentage of fertilization (below 100%), as a percentage of the initial control value. The estimations of motility were subjective, but the figures given represent the judgment of three experienced observers and are believed to give a reasonably fair picture of the activity of the spermatozoa. The results of the tests and examination, given in Table I, show that the fertilizing capacity and motility of the control sperm drop off rapidly along with the decrease in respiratory rate. The sperm in glycine, however, remain highly active and retain high fertilizing capacity, as previously reported(1), although the respiratory rate also drops to a low level. Thus, after one hour the control sperm respire at a higher rate than do the sperm in glycine. The latter, however, exhibit considerably higher motility and fertilizing capacity. By 3 hours the control rate of oxygen consumption has dropped below that of the glycine-sperm. At this time the control sperm are almost all motionless and have very slight fertilizing capacity while that of the glycine-sperm remains high. At 9 hours the respiratory rate of the glycine-sperm has dropped to about 10% of its original value while fertilizing capacity and motility remain near their value at the start of the experiment.

These results are typical of the other experiments. A rather prolonged experiment is illustrated in Fig. 3. In this graph the cumulative values for oxygen uptake are given for a period of 34 hours for the sperm in glycine. The control sperm, in ordinary sea water, showed the secondary rise in rate of oxygen uptake starting at about 9 hours. The vessel was removed at 16 hours at which time tests of fertilizing capacity and motility showed the spermatozoa to be dead and mostly disintegrated with large numbers of bacteria present. In another control containing penicillin (50 units per ml) the secondary rise did not occur. Nevertheless, upon testing the spermatozoa at 16 hours they were found to be immotile and non-fertilizing. The glycine-

TABLE I. Results of Tests of Fertilizing Capacity and Examination of Motility for Respiration Experiment Illustrated in Fig. 2. (See text).

Time of testing, hr	Sperm suspended in	Relative fertilizing capacity, %	Degree of motility, %
1	Sea water	10 to 20	50
1	M/20 glycine	100	75 to 100
3	Sea water	<0.5	<0.1
3	M/20 glycine	100	75 to 100
9	Sea water	0	<0.01
9	M/20 glycine	90 to 95	50 to 75

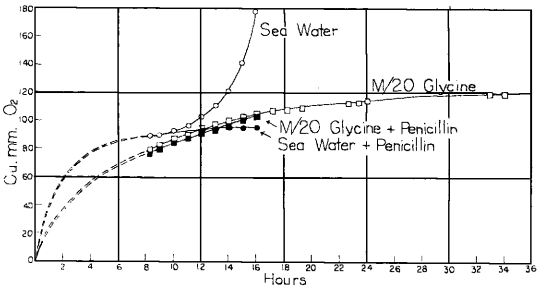

FIG. 3.

Oxygen uptake of senescing spermatozoa of *Lytechinus pictus* in sea water with and without glycine (M/20), and with and without penicillin (50 units per ml). Suspension: approximately 2×10^8 spermatozoa in 3 ml. Temp., 22°C.

sperm, with or without penicillin, showed no secondary rise in the respiratory rate, which from the 16th to the 34th hour, remained at the rather low average value of 0.4 cu mm per hour per 10^8 spermatozoa (*i.e.*, about 2.5% of the rate during the first hour). The motility of the latter at this time (34 hours) was about 50% and fertilizing capacity, compared with freshly collected sperm, of the order of 25%. It is of interest to note, too, that bacteria failed to grow, even without penicillin present, after 34 hours in the glycine-sperm suspension. This is understandable on the basis that no substrate is available for their growth until the sperm start to disintegrate. A similar explanation has been employed in studies(7) on the life span of unfertilized eggs.

It is clear from these experiments that the high initial rate of respiration exhibited by freshly diluted sperm is not a necessary concomitant of high motility and high fertilizing capacity. The spermatozoa in amino acid solution exhibit high motility and fertilizing capacity for a prolonged period during which their respiratory rate has dropped to a very low value. It might be supposed that the amino acid is metabolized in some nonoxidative manner or that it greatly enhances, somehow, the energetic efficiency of the normal oxidative processes. Previous experiments(1) showed no significant utilization of glycine by the sperm during prolonged incubation nor any significant production of ammonia therefrom. Further tests with carboxyl C^{14}-labelled glycine, kindly supplied by Dr. Henry Borsook, showed a negligible amount of decarboxylation.

In this experiment the weight and radioactivity of the carbon dioxide absorbed in the alkali well of a large respiration flask was determined for 20 ml of a 1% sperm suspension (5×10^9 spermatozoa per flask) in 0.05 molar glycine, containing the C^{14}-labelled glycine, for 4 and 8 hours of incubation. In the 8 hours a total of 639 cu mm of oxygen were consumed. From the C^{14}-content of the CO_2 absorbed in the alkali well approximately 0.0026 mg of the 75 mg of glycine present were decarboxylated; *i.e.*, about 0.0035%. A total of 3.8×10^{-5} moles of CO_2 (determined as $BaCO_3$) was absorbed in the alkali well of the respiration flask in the 8 hour period. Of this amount approximately 0.1% was derived from the glycine. The figures for the flask run for 4 hours were of the same order of magnitude.

It is noteworthy that the initial rate of oxygen consumption of the glycine-sperm is distinctly lower than that of the controls. This inhibition in rate is found to be even more marked when higher concentrations of the amino acid are employed. Fig. 4 illustrates an experiment in which glycine is tipped into the vessels from the side arm, at 30 minutes after the start, to give final concentrations of 0.16, 0.05, 0.01 and 0.002 molar. In the two higher concentrations the rate of

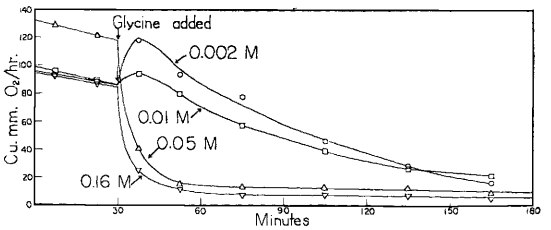

FIG. 4.

Action of glycine on rate of oxygen uptake of spermatozoa of *Lytechinus pictus*. At indicated time 1 ml of appropriate concentration of glycine in sea water added, from side-arm, to a suspension of approximately 9×10^8 spermatozoa in 2 ml of sea water, to give final concentrations listed. 50 units per ml penicillin present in all solutions. Temp., 22°C. See text for further data.

7. Tyler, A., Ricci, N., and Horowitz, N. H., *J. Exp. Zool.*, 1938, v79, 129.

oxygen uptake drops considerably upon the addition of the glycine. In the two lower concentrations there is no initial depression in rate but rather a slight temporary rise (dilution effect). In this experiment, which ran for 18 hours (with 50 units per ml of penicillin present) the total oxygen uptake, relative fertilizing capacity, motility and number of bacteria at the end were as follows:

conc. of glycine	0.16 M	0.05 M	0.01 M	0.002 M
cu mm O_2 consumed	127	186	239	221
fertilizing capacity (%)	50- 75	50- 75	0	0
degree of motility (%)	75-100	75-100	0	0
bacteria per ml	420	240	760	820

The number of bacteria present is negligible as far as their contribution to the respiration is concerned. The total oxygen uptake at the end of the 18 hour period is much lower for the spermatozoa in the stronger than in the weaker glycine solutions. However, in the weaker glycine solutions respiration had almost ceased at this time while in the stronger solutions it averaged 4 to 5 cu mm per hour. In time, then, the higher totals for the latter would be obtained, as in other experiments employing concentrations of amino acid that give prolonged survival of the spermatozoa. The main points, to be noted in this experiment, are the depression of respiration and the continuing low rate in glycine solutions that maintain fertilizing capacity and motility.

Tests for anaerobic metabolism. In view of the low rate of oxygen uptake of the rather highly active sperm during the period of extended life span in amino acid solutions it seemed desirable to examine the possibility of the occurrence of appreciable glycolysis in the treated sperm. Under aerobic conditions sea urchin sperm produce relatively insignificant amounts of lactic, or other, acid and even less under anaerobic conditions with or without added sugar(8,9). In the present experiments determinations were made of anaerobic acid-production by glycine-treated sperm. The experiments were performed using ordinary sea water and an N_2 atmosphere in the vessels and also with bicarbonate enriched sea water in a 5% CO_2 in N_2 atmosphere. Penicillin (50 units per ml) was present in all the solutions. Only very slight CO_2-production was obtained. Thus, 10^9 spermatozoa in 4 ml of sea water gave rise to a total of 20 cu mm of CO_2 in 6 hours, and in glycine the total was less rather than more. The addition of glucose had no noticeable effect. In view of the absence of any indication of glycolysis the results of examination of the spermatozoa removed from the anaerobic vessels proved rather startling. While the spermatozoa in sea water were completely inactive and non-fertilizing those removed from the glycine flasks were highly active (50 to 75%) and capable of fertilization after 6 hours of anaerobiosis.

Anaerobic motility induced by glycine. While the activity observed after removal of the glycine-treated spermatozoa from the vessels might be attributable to a reactivation in the presence of oxygen it seemed desirable to examine the possibility of anaerobic motility. A gas tight chamber for microscopic examination was, therefore, constructed.† Two hanging drops of sperm suspension, one in 0.05 M glycine-sea water and the other in ordinary sea water were introduced into the chamber which was then filled with purified N_2. Determinations of the degree of anaerobiosis attained at various times after filling the chamber were not made. The residual O_2 is removed by the spermatozoa themselves. Under the conditions of the experiments the spermatozoa in the sea water drop usually became motionless in one-half hour after filling the chamber with nitrogen. A large fraction of this time was undoubtedly required for removal of oxygen from the chamber and the hanging drops. In striking contrast to the short duration of motility of the spermatozoa in the sea water drop, those in the glycine-containing drop remained highly active for periods of 4 to 6 hours.

Although it is difficult to put the following observations on a quantitative basis, we have

8. Spikes, J., *Am. Naturalist*, 1949, v83, 285.
9. Rothschild, Lord, *J. Exp. Biol.*, 1948, v25, 353.

† We are indebted to Mr. James H. Berrian for the construction of this chamber.

on several occasions noted: first, that as anoxia develops, the spermatozoa in ordinary sea water swim extremely actively for a short period before becoming motionless; and secondly, that the spermatozoa in sea water containing glycine swim *more* actively under anaerobic than aerobic conditions.

It must be concluded that the addition of glycine to the sea water converts an aerobic cell into one capable of sustained mechanical activity under anaerobic conditions. The results reported in the previous section make it unlikely that there is sufficient glycolytic activity to account for the observed motility. In addition determinations of possible ammonia production and urea formation under anaerobic conditions yielded no significant quantities of these.

Discussion. The present results show that sea urchin spermatozoa suspended in sea water containing an amino acid remain highly motile while consuming oxygen at a very low rate compared with the initial rate of control sperm in ordinary sea water. The rate of oxygen uptake of a sperm suspension does not appear to be commensurate with the motility of the spermatozoa in the suspension. This implies that only a fraction of the oxygen uptake of a freshly diluted suspension is concerned with the liberation of the energy necessary for movement. This possibility is supported by the observation(9) that a considerable reduction in respiration can be effected with CO/O_2 mixtures in the dark without any corresponding reduction in motility. However, it is also clear, from the unexpected finding of anaerobic motility in the presence of glycine that sea urchin spermatozoa are not exclusively dependent upon a supply of oxygen for prolonged activity. As there is, even in the presence of glycine, an insignificant amount of anaerobic glycolysis or CO_2 production, negligible ammonia production, and no indication of urea formation, it seems improbable that glycine is utilized anaerobically as a substrate. Previous evidence(1) along with the present labelled glycine experiments make it very unlikely that it is so utilized under aerobic conditions. This view is supported, too, by the fact that, though the action of glycine is proportional to its concentration, those concentrations at which its effect is not optimal (*e.g.* 0.01 molar) would probably be high enough to achieve optimal enzyme saturation if the amino acid were acting as a substrate.

Since the amino acid does not appear to act as a substrate, it follows that its presence enables the spermatozoa to utilize their endogenous substrate for motility and maintenance of fertilizing capacity with more than normal efficiency. After motility has ceased in a suspension of sea urchin spermatozoa, the sperm still contain oxidizable material (9). Together with the present results, this means that spermatozoa do not normally die of substrate exhaustion.

Further experiments are needed to elucidate the mode of action of amino acids and peptides in achieving these results. One possible explanation centers around the role of trace metals, such as copper and zinc, in sea urchin sperm metabolism(10). The amino acid or peptide might exert their effect by forming complexes with trace metals in the medium. Experiments are in progress to investigate this and other possibilities.

Summary. 1. The metabolism of sea urchin spermatozoa has been examined in sea water containing amino acids which, as had been previously shown, greatly extend the functional life span of these cells. 2. The oxygen uptake of spermatozoa in sea water containing amino acid resembles that of spermatozoa in normal sea water in that a high initial rate is followed by a steady decrease. 3. In the presence of amino acid, however, the initial rate is lower than that of the controls, the degree of inhibition increasing as the concentration of the amino acid is increased. 4. The low rates of oxygen uptake attained after a period of senescence are significantly higher in the presence of amino acid than in the controls. 5. Suspensions of spermatozoa in sea water show a secondary rise in O_2 uptake shortly after a period of extremely low, or non-existent, oxygen uptake following loss of motility and fertilizing capacity. 6. This

10. Rothschild, Lord, and Tuft, P. H., *J. Exp. Biol.*, 1950, v27, 59.

secondary rise is much delayed in the presence of amino acid, corresponding to the extended period of motility and fertilizing capacity. 7. The secondary rise is associated with the growth of bacteria as the spermatozoa die and disintegrate. 8. The addition of penicillin (50 units per ml) is efficacious in checking bacterial growth and the secondary rise in oxygen uptake in suspensions of senescing spermatozoa. 9. In the presence of amino acid, motility and fertilizing capacity can be maintained near their original level for many hours after the rate of oxygen uptake has dropped to less than 5% of the initial rate. 10. Previous evidence that the spermatozoa do not metabolically utilize the added amino acid to any appreciable extent is further supported by the results of experiments with carboxyl C^{14}-labelled glycine. 11. The total oxygen consumption of spermatozoa in the presence of amino acid exceeds that of the controls, which means that the death of the spermatozoa under ordinary conditions is not due to substrate exhaustion but rather to the inability to utilize fully their endogenous substrate. 12. While sea urchin spermatozoa in sea water are quickly immobilized by lack of oxygen, the presence of amino acids enables them to remain highly active for as long as 6 hours at room temperature. 13. No significant glycolysis, CO_2 production, production of ammonia or urea formation is found to occur anaerobically in the presence or absence of amino acid and glycolyzable sugar.

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Antibiotics in Mature Fowl Nutrition.* (18387)

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Olcese, Couch and Lyman(1) reported that the hatchability of eggs from hens fed a diet low in vit. B_{12} decreased to zero in 2 to 4 weeks and further that the hatchability of eggs from hens fed this diet, supplemented with APF concentrates, decreased about the ninth or tenth week of the experimental period. Subsequently Olcese and Couch(2) showed that the injection of crystalline B_{12} into eggs from hens fed a diet low in the vitamin promoted normal hatchability from the sixth through the eighth week but failed to do so after the ninth week in a 17-week experiment. The above-mentioned reports indicate that the hens were depleted of an unidentified factor which was required for normal embryonic development about the ninth or tenth week of the experimental period, since B_{12} was supplied either in the form of an APF concentrate or as the crystalline vitamin. Couch *et al.*(3) in a later report showed that liver fraction "L" contained the unidentified hatchability factor since the feeding of this substance maintained normal hatchability for a period of 16 weeks; whereas, hatchability of eggs from hens given weekly injections of B_{12} decreased after the twelfth week of the test. Cravens and Halpin(4) reported earlier that Liver "L" and fish solubles contained an unidentified factor required by the hen for egg production and hatchability. A number

* Supported in part by Project RG 1862, Division of Research Grants and Fellowships, U. S. Public Health Service, National Institutes of Health, Bethesda, Md.

1. Olcese, O., Couch, J. R., and Lyman, C. M., *J. Nutrition,* 1950, v43, 71.

2. Olcese, O., and Couch, J. R., *Poultry Sci.,* 1950, v29, 612.

3. Couch, J. R., Olcese, O., Sanders, B. G., and Halick, J. V., *J. Nutrition,* 1950, v42, 473.

4. Cravens, W. W., and Halpin, J. G., *J. Nutrition,* 1949, v37, 127.